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CORRELATION BETWEEN YIELD AND PROTEIN CONTENT OF WHEAT AND BARLEY IN RELATION TO BREEDING[1]

By K. W. Neatby[2] and A. G. McCalla[3]

Abstract

High yielding varieties of wheat and barley have a marked tendency to be constitutionally low in protein content. While varieties characterized by moderately high yield and high protein content are known, it is doubtful whether the maximum possible yield can be combined with maximum possible protein. The problem of breeding hard red spring wheats is complicated by this relation, while breeding soft wheats and low protein malting barleys is simplified.

Introduction

Regardless of whether cereals are bred for human consumption or as food for livestock, yield and protein content are characters of great importance. These two characters, in common with many others, are difficult to manipulate in breeding work because they are inherited in a complex manner, and their expression is notoriously subject to environmental influence. There is a general tendency for conditions conducive to high yield to depress protein content, and for those which result in low yield to increase it. Exceptions to this general rule do occur, however, particularly when different regions or soil types are included in the comparisons made. A study of the relation between yield and protein content of Red Bobs and Marquis wheat at Edmonton was made by Malloch and Newton (1). In 1930 and again in 1931, 50 rod-row samples were taken from one wheat field, and yield and protein determinations made. The relation as expressed by the correlation coefficient was −.68 for Red Bobs in 1930 and −.42 for Marquis in 1931.

Another illustration of the principle suggested by the results of Malloch and Newton is afforded by the rod-row tests of rust resistant hybrids conducted at the Dominion Rust Research Laboratory, Winnipeg, from 1932 to 1936 inclusive. In Fig. 1 the mean yield is plotted against the mean protein for each year. Detailed data are recorded in Table II. But for the year 1933, a very close negative relation would be indicated. However, the 1933 results must be disregarded since the protein determinations were not made on the rod-row material which provided the yield data, but on increase plots grown on a field about half a mile away from the rod-row test. In the other four years both protein and yield data were derived from the same plots.

[1] *Manuscript received October 2, 1937.*
Contribution from the Department of Field Crops, University of Alberta. Issued as paper No. 129 of the Associate Committee on Grain Research of the National Research Council and the Dominion Department of Agriculture.
[2] *Professor of Field Crops.*
[3] *Research Assistant, Associate Committee on Grain Research, University of Alberta.*

An examination of yield and protein data secured from cereal variety trials over a period of years at the University of Alberta suggested to the authors that genetical differences in the yielding ability of varieties might be associated with protein content. If varieties characterized by high yield tend to be low in protein, the improvement of malting barley and soft wheat by breeding methods would be simplified, but breeding hard red spring wheats would be seriously complicated. The only published data bearing on this question which have come to the attention of the authors are those of Waldron (2).* He found the correlation coefficient calculated from the yield and protein content of 25 varieties grown in a comparative test to be −.556.

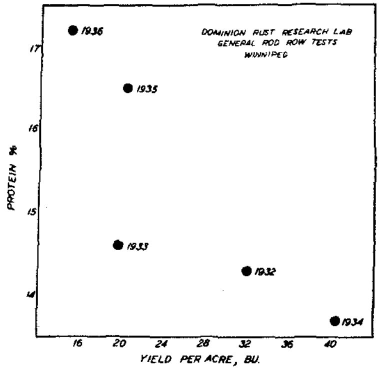

FIG. 1. *The relation between yield per acre and protein content of wheat at Winnipeg for the period 1932 to 1936.*

The publication of the data assembled in this paper has been made possible through the kind permission of the following: Dr. B. B. Bayles, Bureau of Plant Industry, United States Department of Agriculture; Dr. L. H. Newman, Cereal Division, Dominion Department of Agriculture; Drs. C. H. Goulden and R. F. Peterson, Dominion Rust Research Laboratory; and the Associate Committee on Field Crop Diseases of the National Research Council and the Dominion Department of Agriculture.

Hard Wheats

The co-operative test of rust resistant varieties of wheat conducted by the Associate Committee on Field Crop Diseases has been located at various points in each of the three prairie provinces for several years. Yield and

**The results published by A. G. O. Whiteside (Can. J. Research, C, 14: 387-393. 1936) were unfortunately overlooked when this paper was prepared.*

protein data for the years 1933, 1934 and 1936 are summarized in Table I. In this and other tables, yields are expressed in terms of bushels per acre, and protein contents are calculated on the basis of a moisture content of 13.5%. The correlation coefficients vary from $-.07$ to $-.83$ in the 1933 series. At certain stations, notably Morden and Saskatoon, the relation between high yield and low protein content is very close. In 1933 each variety was seeded in four replicate plots. Under such circumstances observed differences in yield may not be entirely genetical, since identical conditions for all varieties cannot be secured. With this fact in mind the correlation between the general means of yield and protein for all stations was determined. The coefficient obtained, $-.55$, is almost certainly due largely to genetical causes. It is true that environmental effects cannot be entirely eliminated, since a certain degree of interaction between variety and station for each of the two characters in question is likely to occur. The relative importance of genetical and environmental effects on the correlation coefficient cannot be determined without knowledge of the protein content of individual plots. This information is, unfortunately, lacking.

The practical significance of the above results is indicated by the regression coefficients and the figures in the last four columns in the table. It is clear that if selection was based on yield alone the general level of protein content would be lowered. Similarly, if protein content was emphasized, the selection would automatically favor low yield. Swan River is the only station at which the relation fails to hold. The figures based on the 1933 general means are very significant. The difference of 7.9 bushels per acre between the five varieties with the highest protein and the five with the lowest is, from a breeding point of view, of substantial importance.

The data for 1934 are similar, though the relations are not quite so close. In 1936 the magnitude of the correlation coefficients was greatly reduced. This reduction is without a doubt partly due to the fact that protein content has been stressed in the choice of varieties for inclusion in the co-operative test. An examination of the records has revealed the fact that several varieties which were outstanding from the point of view of yield during the period 1931–33 were discarded on account of low protein and inferior loaf volume. It is necessary to point out, however, that the 1936 results are not strictly comparable with those of 1933 and 1934, since in 1936 the mean yield was low and the mean protein content high, owing to unusually dry conditions.

The results of three spring wheat and two winter wheat tests are summarized in Table II. In the University of Alberta tests, the correlation coefficients do not indicate a very close relation between protein content and yield, except in the case of the 1930 results. However, in all years but 1936 the differences in yield between the five varieties with highest and the five with lowest protein are considerable. Likewise, the difference in protein content of the five highest yielding and the five lowest yielding varieties are large enough to deserve careful attention.

TABLE I

RELATION BETWEEN YIELD AND PROTEIN, CO-OPERATIVE TEST OF RUST RESISTANT VARIETIES

Place	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
			Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
1933. 36 varieties										
Edmonton, Alta.	65.7	15.2	10.8	5.3	−0.64	−0.073	57.7	68.4	14.6	16.2
Fallis, Alta.	43.8	12.4	15.0	8.0	− .50	− .076	33.0	45.4	12.0	13.5
Lacombe, Alta.	53.5	15.8	11.6	4.1	− .71	− .073	44.2	60.1	15.3	16.6
Indian Head, Sask.	50.9	15.6	10.3	4.6	− .58	− .081	44.7	56.1	15.0	16.3
Saskatoon, Sask.	16.5	15.6	14.2	4.3	− .78	− .221	13.6	19.4	14.9	16.5
Scott, Sask.	6.9	14.9	21.0	4.2	− .43	− .186	5.2	7.9	14.6	15.2
Swift Current, Sask.	22.5	15.8	12.1	4.6	− .15	− .040	22.8	24.3	15.2	15.8
Brandon, Man.	37.2	14.4	10.2	5.2	− .45	− .088	36.5	37.5	14.1	14.8
Morden, Man.	27.1	16.4	12.9	4.4	− .83	− .171	21.5	31.9	15.5	17.2
Swan River, Man.	42.4	11.0	14.4	12.7	− .07	− .015	44.5	43.8	11.6	11.6
Winnipeg, Man.	17.1	14.1	11.7	5.7	− .71	− .282	14.7	19.0	13.6	14.8
Mean	33.7	15.4	9.0	4.2	− .55	− .118	29.6	37.5	14.8	16.5
1934. 25 varieties										
Edmonton, Alta.	53.6	14.9	9.4	3.7	− .10	− .011	51.9	60.1	14.8	15.1
Fallis, Alta.	18.4	9.7	13.9	4.7	− .77	− .175	15.6	21.3	9.0	10.2
Lacombe, Alta.	26.5	15.0	13.6	5.0	− .68	− .143	22.9	32.7	14.2	15.7
Indian Head, Sask.	32.6	14.9	11.2	4.1	− .31	− .052	30.6	35.8	14.4	15.5
Saskatoon, Sask.	26.7	15.9	8.0	2.9	− .30	− .065	26.6	27.9	15.9	16.0
Swift Current, Sask.	20.0	16.9	7.9	2.8	+ .11	+ .034	20.2	19.9	17.1	16.9
Brandon, Man.	33.9	15.1	7.7	3.5	− .50	− .100	31.7	35.8	14.8	15.5
Morden, Man.	34.0	16.1	6.9	3.4	− .43	− .100	32.4	36.4	15.7	16.4
Swan River, Man.	36.3	10.0	10.8	8.9	− .07	− .015	33.9	37.4	9.7	10.0
Solsgirth, Man.	24.5	13.6	13.7	3.8	− .31	− .049	24.3	27.1	13.1	13.6
Mean	30.3	14.1	5.7	3.4	− .50	− .136	29.3	30.9	13.8	14.6

TABLE I—*Concluded*

RELATION BETWEEN YIELD AND PROTEIN, CO-OPERATIVE TEST OF RUST RESISTANT VARIETIES—*Concluded*

Place	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
			Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
1936. 22 varieties										
Edmonton, Alta.	30.4	15.2	11.7	6.0	− .39	− .100	28.4	31.4	14.4	15.3
Fallis, Alta.	10.0	15.1	18.4	5.8	− .64	− .308	8.3	11.1	14.8	16.2
Lacombe, Alta.	21.7	16.0	19.3	4.2	− .14	− .023	19.6	22.7	16.0	15.8
Indian Head, Sask.	28.6	15.7	10.2	5.4	+ .11	+ .032	28.7	28.0	15.5	15.7
Saskatoon, Sask.	18.4	18.2	11.3	4.3	− .38	− .141	17.2	19.5	18.1	18.6
Scott, Sask.	6.8	17.3	11.6	4.2	− .35	− .320	6.5	7.4	16.9	17.7
Swift Current, Sask.	10.4	18.8	10.3	3.1	+ .23	+ .126	10.8	10.8	18.9	18.8
Brandon, Man.	26.0	16.6	12.0	4.4	.00	.005	27.0	28.0	16.7	16.7
Morden, Man.	17.3	19.8	11.7	3.8	− .37	− .138	16.8	18.8	19.1	20.1
Solsgirth, Man.	11.8	16.2	13.1	6.0	− .61	− .381	10.3	12.7	15.8	17.2
Winnipeg, Man.	10.1	16.5	22.8	4.9	− .10	− .035	10.7	10.2	16.7	17.1
Mean	17.9	16.8	6.7	3.1	− .25	− .108	17.6	18.4	16.6	17.0

TABLE II

RELATION BETWEEN YIELD AND PROTEIN, MISCELLANEOUS HARD WHEATS

Place	Year	No. of varieties	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
					Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
Rod-row tests, University of Alberta												
Edmonton	1929	33	29.9	16.1	17.5	7.7	−0.65	−0.154	23.9	34.1	15.0	17.9
Edmonton	1930	21	35.6	15.6	16.0	7.1	− .76	− .147	30.3	40.6	15.0	16.9
Edmonton	1931	49	52.3	14.5	12.4	6.3	− .54	− .075	42.3	54.0	14.1	15.9
Edmonton	1932	67	43.2	15.1	10.5	5.3	− .38	− .067	39.9	46.0	14.8	15.8
Edmonton	1933	35	61.3	15.5	10.6	5.4	− .30	− .039	58.2	65.3	15.4	16.1
Edmonton	1936	15	40.8	14.4	13.6	5.5	− .34	− .048	39.2	39.4	13.7	14.6
Fallis (fallow)	1933	12	39.1	11.5	22.4	6.8	− .34	− .031	35.7	39.1	11.2	11.8
Fallis (stubble)	1933	12	18.0	10.5	14.8	8.4	− .60	− .197	17.0	19.2	10.0	10.6
Rust resistant hybrids, Dominion Rust Research Laboratory												
Winnipeg	1932	101	32.0	14.3	11.7	5.1	− .37	− .072	29.7*	35.0*	13.4*	14.6*
Winnipeg	1933	141	19.6	14.6	13.3	5.4	− .42	− .127	17.0*	21.8*	14.0*	14.8*
Winnipeg	1934	124	40.4	13.7	11.2	5.5	− .23	− .038	37.0*	40.8*	13.0*	14.1*
Winnipeg	1935	108	20.4	16.5	19.8	5.3	− .19	− .040	16.3*	20.4*	15.6*	16.3*
Winnipeg	1936	85	15.0	17.2	14.3	4.2	− .16	− .052	12.9*	15.5*	16.9*	17.4*
Drought hybrids and miscellaneous varieties, University of Alberta												
Brooks, Alta.	1936	75	10.0	12.0	24.7	8.0	− .51	− .138	7.5*	11.8*	11.6*	13.0*
Winter wheats, University of Alberta												
Edmonton	1931	46	28.4	15.8	16.9	3.5	− .26	− .031	24.0	31.8	15.4	15.7
Winter wheats, U.S.D.A. western region												
**5 Station mean	1935	24	52.2	9.3	5.7	4.6	− .57	− .081	48.7	54.4	8.9	9.7

* *10 samples instead of 5.*
** *Pullman, Pomeroy and Walla Walla, Washington; Pendleton, Oregon; Newton, Utah.*

Perhaps the most interesting feature of the results of the Dominion Rust Research Laboratory tests is the reduction in magnitude of the coefficients from 1932 to 1936. It is quite possible that this is due, in part at least, to the elimination of high yielding strains on the basis of protein content and of high protein strains on account of low yield.

The test of miscellaneous varieties and hybrid strains conducted at Brooks, Alberta, by the University in 1936 was exposed to conditions of very severe drought. The relation between yield and protein is similar to those already described.

Numerous data on winter wheat varieties are available, but tests in which winter killing occurred cannot be legitimately quoted in the present connection. The data at the bottom of Table II suggest that the relation observed in spring wheats holds also in winter wheats. In the United States Department of Agriculture test, samples from five stations were composited for protein determinations. This procedure virtually eliminates environmental effects and, consequently, the correlation coefficient of $-.57$ is almost entirely due to genetical relations. The magnitude of the regression coefficient indicates, however, that fairly large differences in yield are associated with relatively small differences in protein content.

Early Hard Spring Wheats

The development of early high yielding spring wheats of good quality is engaging the attention of several Canadian plant breeders. It so happens that the areas of Manitoba, Saskatchewan and Alberta chiefly concerned are, in many cases, characterized by relatively infertile soils and more humid conditions than those prevailing on the open plains. Therefore the question of yield in relation to quality assumes considerable importance. A new variety, if it is to be accepted, must be productive and it must be early. Protein content is equally important because the conditions of soil and climate in the northern areas, generally speaking, are not conducive to the production of wheat of high protein content.

The situation with regard to yield and protein content in several comparative trials including new and old early varieties is summarized in Table III. Again there is a definite and, in some cases, a close inverse relation between yield and protein content. In the University of Alberta test, 18 of the 32 varieties were selected from the cross, Reward × Double Cross. The latter variety was selected by Dr. H. K. Hayes of the University of Minnesota from (Marquis-Iumillo) × (Marquis-Kanred). The necessity of compromising between maximum yield and maximum protein is well brought out by the Edmonton test of Reward × Double Cross strains. Selection for maximum yield will almost certainly involve a sacrifice of protein.

The Fallis results are of particular interest, since the soil at Fallis is deficient in nitrogen and sulphur and is typical of large areas for which early varieties are being developed. The high values of the regression coefficients calculated

TABLE III

RELATION BETWEEN YIELD AND PROTEIN, EARLY WHEATS, 1936

Place	No. of varieties	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
				Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
University of Alberta, general series											
Edmonton	32	27.5	14.6	12.2	5.4	−0.35	−0.082	25.2	29.3	13.8	14.7
Fallis	32	8.8	14.8	23.3	7.0	− .54	− .272	6.6	10.3	14.0	15.6
University of Alberta, Reward × Double Cross											
Edmonton	18	27.7	15.5	13.0	4.4	− .70	− .176	26.1	32.1	14.7	16.0
Fallis	18	9.5	14.9	13.4	6.1	− .60	− .323	8.5	10.0	14.5	15.7
Cereal Division co-operative early wheat test											
Beaverlodge	24	42.3	13.3	14.7	7.2	− .54	− .082	37.0	47.9	12.4	13.6
Edmonton	24	25.2	15.4	8.0	7.0	− .40	− .212	24.5	26.8	14.5	15.8
Indian Head	23	23.3	14.3	13.3	8.8	− .56	− .227	21.6	25.9	13.3	15.2
Scott	24	8.6	17.1	9.3	4.6	− .57	− .559	8.1	10.0	16.2	17.7
Swift Current	24	13.6	18.9	5.2	3.1	− .48	− .399	13.0	14.1	18.7	19.4
Ottawa	23	26.7	14.7	11.8	8.1	− .14	− .053	26.6	28.4	14.2	14.8
Mean	22	21.9	15.6	7.2	5.0	− .52	− .257	21.1	22.8	14.8	16.1

on the Fallis tests indicate that the introduction of more productive varieties may result in a more or less serious reduction in protein content.

The same general conclusion can be drawn from the results of the co-operative early wheat test conducted by the Cereal Division of the Dominion Department of Agriculture. The Beaverlodge results are of particular interest, in that the five varieties with the lowest average protein yielded 10.9 bushels per acre more than did the five varieties with the highest average protein content.

Soft Spring Wheats

With a view to determining the adaptability of soft wheats to the gray wooded soil areas of northern and northwestern Alberta, comparative tests of 11 varieties have been conducted at various points by the Department of Field Crops of the University of Alberta. The 1936 results relevant to the subject of this paper are given in Table IV. At every station except Beaverlodge the negative correlation between yield and protein content is high. The low figure for Beaverlodge is undoubtedly due to the fact that varietal differences in yield were small. This is indicated by the low coefficient of variability. The correlation between mean yield and mean protein is extremely high and indicates that within the limits of this experiment the genetical potentialities for protein content are largely determined by the yielding ability of the varieties. It is necessary to point out that two of the varieties, Marquis and Red Bobs, are not soft wheats. However, the position of these two varieties in Fig. 2 indicates that they do not contribute more to

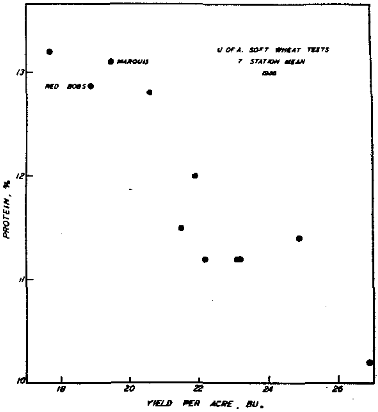

FIG. 2. *The distribution of varieties of soft wheat according to yield per acre and protein content.*

TABLE IV

RELATION BETWEEN YIELD AND PROTEIN, SOFT WHEATS

Place	Year	No. of varieties	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
					Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
University of Alberta tests												
Athabasca	1936	11	6.7	10.2	19.2	10.7	−0.77	−0.660	6.0	7.6	9.4	10.9
Bon Accord	1936	11	9.2	13.7	42.0	9.8	− .86	− .301	6.6	11.9	12.9	14.6
Beaverlodge	1936	11	58.8	11.2	6.7	9.5	− .32	− .086	56.5	59.7	10.7	11.9
Edmonton	1936	11	39.4	13.6	14.8	5.4	− .76	− .121	34.9	43.5	13.0	14.2
Fallis	1936	11	12.8	11.6	20.8	9.5	− .83	− .345	10.8	15.2	10.7	12.5
Mellowdale	1936	11	13.4	9.3	11.7	10.7	− .73	− .456	12.2	14.7	8.5	10.2
Warburg	1936	11	11.8	13.5	20.1	8.9	− .83	− .420	9.6	13.6	12.6	14.5
Mean	1936	11	21.8	11.9	11.7	8.3	− .92	− .357	19.7	23.9	11.0	12.7
U.S.D.A. western region												
Davis, California	1935	15	56.0	9.5	8.0	9.4	− .14	− .028	57.7	54.5	9.8	9.8
1. Three-station composite*	1935	32	59.9	10.8	8.0	8.1	− .56	− .104	53.9	61.0	10.4	11.9
2. Four-station composite*	1935	19	35.7	11.7	7.1	6.9	− .63	− .201	33.0	37.1	11.2	12.2

*1. *Aberdeen, Idaho; Logan, Utah; Bozeman, Montana (Irrigated).*

*2. *Pullman, Pomeroy and Walla Walla, Washington; Pendleton, Oregon.*

TABLE V

RELATION BETWEEN YIELD AND PROTEIN, UNIVERSITY OF ALBERTA BARLEY TESTS

Place	Year	No. of varieties	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
					Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
Edmonton	1932	22	45.9	15.1	19.8	8.1	−0.45	−0.060	37.0	52.1	14.6	16.1
Edmonton	1933	22	37.9	14.6	15.7	5.1	− .30	− .037	37.4	42.7	13.7	15.0
Edmonton	1934	26	58.7	15.0	18.9	11.3	− .75	− .115	48.1	68.9	13.4	16.6
Edmonton	1936	32	34.9	14.5	17.9	7.3	− .41	− .070	26.1	38.3	14.5	15.7
Fallis	1933	15	24.1	10.0	20.7	8.9	− .80	− .144	20.7	29.6	9.1	10.6
Fallis	1934	12	28.5	8.8	31.1	10.7	− .72	− .077	23.4	36.0	8.1	9.7
Athabasca	1936	9	8.6	10.2	18.1	10.3	− .39	− .249	7.5*	9.0*	9.4*	10.2*
Beaverlodge	1936	10	27.2	12.8	13.7	8.6	− .74	− .219	25.9	28.4	12.3	13.3
Bon Accord	1936	10	12.1	12.8	35.1	10.7	− .21	− .068	11.5	12.7	12.8	12.8
Fallis	1936	10	13.4	12.1	16.2	8.2	− .73	− .330	12.3	14.6	11.8	12.5
Mellowdale	1936	10	11.8	10.2	18.3	10.5	− .66	− .328	10.9	12.7	9.8	10.5
Warburg	1936	10	12.0	13.8	20.9	7.0	− .88	− .342	10.2	13.7	13.1	14.6
Mean	1936	9	14.2	11.9	14.6	7.8	− .72	− .323	13.3*	15.5*	11.4*	12.6*

* *4 varieties instead of 5.*

the correlation than do the other varieties. It is interesting that at all stations except Edmonton and Beaverlodge the plots were located on soil characterized by a deficiency of nitrogen, and the regression coefficients are high. Since it is on these soils that the problem of low protein is acute, the practical importance of a negative relation between yield and protein content is obvious.

Except in the case of Davis, California, the United States Department of Agriculture data (Table IV) indicate the same general relation.

Barley

The data in Table V indicate that the relation in the case of barley is almost as close as that observed in the soft wheats. The 1934 Edmonton test is particularly striking. The five varieties with the lowest average protein content yielded 20.8 bushels more per acre than did the five varieties with the highest protein. The difference in protein content of the five highest- and the five lowest-yielding varieties is equally marked.

In the Fallis tests the differences in yield between the high and low protein varieties are relatively enormous.

Only ten varieties were included in the tests conducted at country points. One variety, Regal, was omitted at Athabasca and, consequently, the comparisons made for that point and for the means include only nine varieties; only the four highest and the four lowest, according to yield, and similar numbers, according to protein content, were used in obtaining the figures in the last four columns. The results of these tests are similar to those obtained at Edmonton. The negative relation between yield and protein content is quite apparent, and the difference in yield between the high and low protein varieties is remarkably consistent from one station to another.

Discussion

In consideration of the foregoing facts, it is certain that there is a genetically controlled association between high yield and low protein content in wheat and barley. The correlation coefficient cannot be used safely as an absolute measure of relationship, which may, in some cases, be non-linear, and the reliability of the coefficients may be tempered by the "grouping" of varieties within the swarm. In order to determine the exact degree of relationship, it will be necessary to plan experiments with that particular purpose in mind. It is probable that the degree of association will vary from one cross to another. Despite the inadequacy of the available data from the point of view of the degree of association, its significance in relation to breeding problems is obvious. This is emphasized by the magnitude of the regression coefficients in most of the tests and, in several cases, by the large differences in protein content between the highest and lowest yielding varieties.

In so far as hard red spring wheats are concerned, the aim is maximum yield and maximum protein content. Efforts to attain this end are based on the assumption that high yield and low protein content are not inseparably

associated. That this assumption is justified is indicated by the performance of Thatcher wheat, for example, which is characterized by relatively high yield and high protein content. In consideration of all available facts it seems probable that genes, the direct effect of which is to stimulate yielding ability, indirectly depress the protein content just as environmental factors which promote high yield frequently do so at the expense of protein content. It is probable, however, that numerous genes that promote high protein in the grain are available, and such genes would, theoretically, tend to offset the depressing effect on protein content of the "high yield" genes. If this is the true state of affairs, the importance of exploring the maximum possible number of different crosses is clear.

To illustrate the varied potentialities of different crosses, a comparison has been made (Fig. 3) between Pentad × Marquis and H-44-24 × Reward. These data are taken from the general rod-row test conducted at the Dominion Rust Research Laboratory. A general negative relation between protein content and yield is apparent in Fig. 3, A, B, C, and D. The lines of the cross H-44-24 × Reward (circles) have a tendency to fall in the high-protein

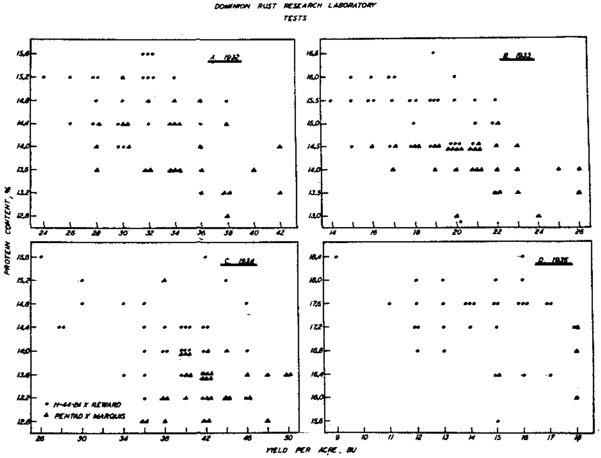

FIG. 3. *The relation between yield per acre and protein content in lines of the crosses H-44-24 × Reward and Pentad × Marquis.*

low-yield area, while the Pentad × Marquis lines (triangles) tend to be high in yield and low in protein. By 1936 (Fig. 3, D) all Pentad × Marquis lines except five (protein data were available for four only) had disappeared from

the test. Doubtless low protein content was an important factor in their disappearance.

To summarize this phase of the discussion it is suggested that the genetical constitution of a high-protein wheat variety may be due to an accumulation of "high protein" genes, or to a paucity of "high yield" genes accompanied by moderate potentialities for high protein.

Throughout this paper it has been assumed that yield and protein content are subject to the same laws of inheritance as are other more readily observable characters. This assumption will hardly be questioned. The differences in general level of yield and protein content for the same material between

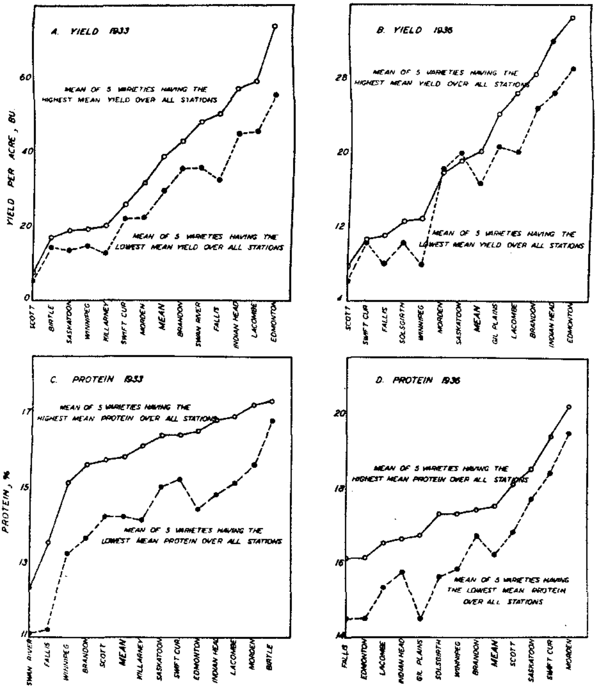

FIG. 4. *The constancy of differences in yield and protein content of wheat varieties at various stations (data from co-operative tests of rust resistant varieties).*

stations are, of course, due to environmental influences such as moisture, soil nitrogen, and general nutritional conditions. The genetical tendency of one variety in the series to be high yielding or high in protein may be so modified by environmental influences that the actual yield or protein content is very low. These influences on the physiology of the plant are not to be confused with the genetical influences on the physiology, since, in any series, the high yielding varieties tend to be relatively high yielding over a wide range of environmental conditions. This is well illustrated by the graphs in Fig. 4, in which the constancy of the genetical differences of rust-resistant varieties from one geographical location to another is shown. If the interaction of these characters with location is very great, then the significance of determinations made at one point may be nullified in so far as other points are concerned. In Fig. 4, A the five varieties giving the highest mean yield for all stations, and the five giving the lowest mean yield, are arranged according to the respective mean values for individual stations in 1933. A similar illustration of the 1936 results is given in Fig. 4, B. The fact that the lines do not cross, except at two stations in 1936, indicates that there is a general agreement between the yield results from station to station. This holds, of course, only in a general way since it is well known that some varieties are more sensitive to regional differences than are others.

The protein data have been arranged in a similar manner (Fig. 4, C and D). The differences are remarkably constant from station to station. This suggests that selection for protein content can be carried out with confidence at any one station. It is possible, however, that certain varieties, or crosses, may behave in an irregular manner under special conditions.

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AGRICULTURAL METEOROLOGY: CORRELATION OF AIR TEMPERATURES IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN WITH LATITUDE, LONGITUDE AND ALTITUDE[1]

By J. W. Hopkins[2]

Abstract

Linear partial regression coefficients of the 18-year average (1917–34) monthly mean air temperature recorded at 43 points in central and southern Alberta and Saskatchewan on latitude, longitude, and altitude were determined for each month of the year. The three series of coefficients each show an independent seasonal trend. The decrease in air temperature with altitude is greatest in summer and least in winter, whereas the gradient associated with longitude is most pronounced in winter and least in evidence in summer. The influence of latitude is likewise most pronounced in winter, but shows two minima, in spring and autumn respectively. The monthly regression equations account for most of the variance of the station averages, and hence provide a reasonably satisfactory graduation of the climatological temperature gradients characteristic of this area at different seasons of the year.

These regression equations could not, however, be applied satisfactorily to the monthly averages for individual years, owing to greater local variation. Additional equations were therefore determined from the records for 1935 at 27 stations in the sub-area bounded by the 50th and 52nd parallels and the 104th and 108th meridians. The results suggest that further additions to the number of stations would still be desirable, and that if this was effected a fairly accurate graduation should be possible within this district, even in individual years.

Introduction

According to Irwin (3, pp. 269 *et seq.*) the question of the adequacy of the number of meteorological stations in any area has been subject to little critical study. Irwin points out that the number of stations per square mile, or its reciprocal, "is really quite an inadequate criterion, for in some regions of a given size weather conditions will be almost uniform, in other regions of the same size they will vary greatly."

One aspect of this subject was touched upon in a previous study by the present writer (2), in the course of which the irregular variation (as distinguished from consistent differences between years and between places) in the monthly totals of precipitation recorded at meteorological stations in central and southern Alberta and Saskatchewan was determined, and the theoretical number of stations required to reduce such irregular or random variation in the district averages to specified levels computed.

With respect to the number of stations required in any area to provide a satisfactory indication of positional effects, Irwin (3) suggests that "Perhaps the only adequate criterion is that any meteorological variate (say rainfall) in which we are interested should in any one station be capable of prediction from the values of the same variate at other neighboring stations. For this purpose it is convenient if areas can be selected sufficiently small for the

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[2] *Statistician, National Research Laboratories, Ottawa.*

regression of the variate on latitude, longitude and altitude to be effectively linear within each such area, and with a sufficient number of stations within the area to enable the regression line to be calculated with an assigned accuracy." A rather different meteorological application of the method of linear regression has recently been made by Schumann (6) in determining the average increase in error of interpolation of monthly rainfall values in South Africa with increasing distance of the point of interpolation from the control stations. His treatment does not, however, take into account the co-ordinates of position and altitude.

In considering the method outlined by Irwin, a distinction may be made between two different types of meteorological data: (a) Climatological averages, specifying the average weather conditions over a period of years; (b) Records descriptive of the weather within the given area during a specified interval of time, such as a particular growing season or portion thereof. Within any climatic zone, (a) may be expected to be more stable than (b), and hence to be capable of specification to an equal degree of accuracy by a coarser network of stations.

In the following sections of this paper, the method is applied to the mean monthly air temperatures at stations in central and southern Alberta and Saskatchewan, which from the meteorological point of view may be expected to provide a homogeneous area, since they are free from major physiographic barriers or large bodies of water which might affect the atmospheric circulation. Linear correlations are determined for the 18-year averages, 1917–34, for each month, permitting a numerical summary of the influence of position and altitude on mean air temperature within the area. The agreement between the actual and linearly graduated values then provides a criterion of the homogeneity of the area represented and of the adequacy of the meteorological network in specifying the temperature characteristics of the area. Finally, the additional variability encountered in considering the results for an individual season is examined quantitatively.

Observational Data

The temperature data used were in all cases extracted from the Monthly Record published by the Meteorological Service of Canada (5). A series of 43 stations in the area designated was found to have continuous or nearly continuous records for the 18-year period 1917–1934. In the earlier years, some gaps occurred, which were filled by the substitution of observations taken at neighboring stations, not otherwise included in the series. Fig. 1 shows the location of the 43 stations, the latitude, longitude and altitude of which will be found in Table I. Table II gives the 18-year average of mean temperature (*i.e.*, mean of daily maximum and minimum) by calendar months for each station and also, at the foot of the columns, the average and standard deviation of the values for all 43 stations for each month. The means illustrate the well-known annual progression of temperature from a minimum, in this case of 8.0° F., in January to a maximum of 63.7° in July. It is to be

FIG. 1. *Location of meteorological stations providing 18-year temperature averages.*

noted that the standard deviation, indicative of the differences between stations in respect of the 18-year average of monthly mean temperature, also shows a definite seasonal trend, being greatest in winter and least in spring and autumn.

Analysis of Climatological Series

From the data in Tables I and II, the partial regression coefficients b_1, b_2 and b_3 of mean temperature on latitude, longitude and altitude were determined by the method of Least Squares. The Normal Equations to determine b_1, b_2 and b_3 for January were:

$$\begin{aligned} 251{,}075\, b_1 + 49{,}761\, b_2 - 1{,}041{,}367\, b_3 &= -11{,}548.5 \\ 49{,}761\, b_1 + 2{,}455{,}745\, b_2 + 5{,}852{,}747\, b_3 &= 50{,}976.2 \\ -1{,}041{,}367\, b_1 + 5{,}852{,}747\, b_2 + 24{,}320{,}901\, b_3 &= 173{,}459.6 \end{aligned}$$

TABLE I

LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS

Station	Latitude north of 49th parallel, min.	Longitude west of 101st meridian, min.	Height above sea level, ft.
Alberta			
Alix	203	730	2585
Bassano	107	688	2625
Calgary	122	782	3540
Calmar	255	770	2200
Edmonton	273	750	2158
Gleichen	112	723	2952
Harmattan	165	803	3500
High River	95	772	3394
Hillsdown	192	755	2940
Lacombe	208	764	2783
Lethbridge	43	711	2961
Lundbreck	34	788	3918
Macleod	44	744	3128
Medicine Hat	61	577	2144
Olds	165	785	3413
Pekisko	82	807	4721
Perbeck	178	725	2850
Ranfurly	269	638	2250
Strathmore	123	743	3160
Saskatchewan			
Anglia	154	430	1861
Battleford	221	440	1620
Chaplin	88	340	2202
Fort Qu'Appelle	107	168	1600
Indian Head	88	160	1924
Kamsack	154	54	1445
Klintonel	38	473	3500
Melfort	232	216	1518
Moose Jaw	81	275	1860
Muenster	192	240	1888
Nashlyn	12	509	3100
Pilger	205	249	1785
Prince Albert	250	285	1432
Qu'Appelle	91	176	2147
Regina	87	217	1884
Rosthern	220	320	1672
St. Walburg	276	489	2050
Saskatoon	195	330	1600
Scott	202	466	2164
Shaunavon	37	442	3010
Swift Current	80	405	2440
Waseca	246	509	2105
Whitewood	78	75	1973
Yellow Grass	49	189	1899

The equations for the eleven other months differ only in the substitution on the right-hand side of the successive trios of products:

−8,946.6	−7,925.3	−2,596.1	−238.7	−1,622.4	−2,716.0
44,812.4	26,956.8	5,733.0	−9,306.3	−18,332.8	−14,700.7
144,407.8	85,699.6	4,237.4	−47,323.0	−65,805.9	−51,748.9

−2,998.0	−2,587.6	−3,459.7	−6,117.0	−9,056.8
−13,990.5	−10,374.1	4,300.1	21,281.5	35,197.7
−46,667.7	−34,908.0	11,178.7	69,882.9	122,253.0

TABLE II

MEAN TEMPERATURE (°F.), 1917–1934, BY MONTHS

Station	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Alberta												
Alix	9.9	14.0	24.0	39.7	50.4	57.4	62.8	60.5	49.9	39.3	25.8	11.6
Bassano	14.4	17.4	2 .7	41.6	53.1	60.7	6 .9	64.4	53.7	42.4	28.7	15.7
Calgary	17.3	20.6	26.7	39.6	50.3	57.2	62.8	60.8	51.2	41.3	29.2	17.4
Calmar	8.6	13.4	21.6	37.6	49.1	55.6	60.1	58.0	48.6	3 .1	24.7	10.6
Edmonton	8.8	14.2	22.7	39.5	51.2	57.6	62.2	59.7	49.9	39.4	25.1	10.6
Gleichen	12.2	16.4	25.2	39.7	50.7	58.3	64.1	61.5	50.	40.2	26.7	14.2
Harmattan	11.7	15.8	22.1	36.3	46.3	53.7	57.9	55.8	46.3	36.9	24.8	12.7
High River	17.8	19.4	26.1	37.5	47.5	54.1	60.0	57.7	48.7	39.7	29.2	17.4
Hillsdown	13.3	16.5	24.6	39.1	50.4	56.8	62.7	59.3	49.4	39.9	26.8	14.4
Lacombe	9.7	14.2	22.4	38.2	49.1	56.3	61.5	59.1	49.2	39.0	25.2	11.0
Lethbridge	19.4	21.1	28.6	40.8	50.9	58.7	64.5	6 .4	52.2	43.1	31.2	19.6
Lundbreck	16.5	17.9	24.4	35.4	44.6	52.0	57.9	55.8	46.8	38.8	28.0	17.2
Macleod	20.6	22.3	30.2	41.8	52.4	60.2	66.4	64.1	53.8	45.0	32.2	20.7
Medicine Hat	16.4	19.7	29.9	44.8	56.7	64.9	70.9	67.3	55.7	45.1	31.2	17.8
Olds	13.7	16.7	23.4	38.1	47.8	51.8	59.7	57.7	48.0	38.9	26.2	14.6
Pekisko	17.5	19.1	24.1	34.2	43.1	50.1	55.7	53.9	45.3	38.2	28.2	17.1
Perbeck	10.8	14.6	23.1	38.8	50.1	57.2	63.2	60.1	49.5	38.8	25.1	11.9
Ranfurly	4.7	9.4	18.9	37.9	51.1	57.7	62.7	60.3	49.8	37.8	22.9	7.1
Strathmore	13.1	15.9	24.6	38.9	49.8	57.3	62.9	59.8	49.9	39.4	26.8	14.0
Saskatchewan												
Anglia	4.5	8.2	19.6	38.0	51.4	60.1	65.1	62.4	51.1	38.4	23.5	7.7
Battleford	2.7	7.9	19.3	38.6	52.8	60.6	65.3	63.0	52.4	39.8	23.6	6.4
Chaplin	7.3	10.9	23.2	38.8	51.9	61.4	66.3	63.5	52.6	39.1	24.7	9.4
Fort Qu'Appelle	2.3	7.6	19.6	38.4	51.4	60.6	66.1	64.2	53.1	39.9	23.2	7.1
Indian Head	2.3	7.5	18.8	36.8	50.6	60.1	65.2	62.6	51.5	38.4	22.3	6.7
Kamsack	− 2.6	1.7	14.6	35.8	51.1	59.7	63.9	61.4	51.3	37.9	20.3	2.6
Klintonel	12.5	15.2	22.8	37.3	48.6	57.1	63.2	60.9	49.9	39.2	26.4	14.4
Melfort	− 1.4	4.0	15.5	35.2	50.3	59.2	63.5	60.5	50.3	37.0	19.5	5.2
Moose Jaw	8.4	12.4	23.9	40.2	53.4	62.6	68.0	65.3	54.3	41.5	26.6	11.8
Muenster	− 1.6	3.9	15.2	35.6	49.6	58.2	62.8	60.0	49.9	37.3	20.2	3.1
Nashlyn	8.4	12.8	23.0	39.8	51.1	59.8	66.6	63.8	51.6	40.2	24.5	10.1
Pilger	− 0.3	5.4	15.7	36.1	50.9	59.3	64.0	61.9	51.4	38.9	21.1	4.5
Prince Albert	0.4	6.1	16.8	36.9	51.7	59.9	64.9	61.8	51.4	39.4	21.8	6.2
Qu'Appelle	4.5	8.8	20.2	37.6	51.2	60.1	64.9	62.7	52.3	39.6	23.4	8.4
Regina	3.9	8.3	20.3	38.4	52.1	61.2	66.2	63.6	53.1	39.2	22.8	7.8
Rosthern	− 0.4	5.3	16.4	36.9	51.9	60.2	64.9	62.1	51.0	38.3	21.2	5.5
St. Walburg	− 2.4	2.8	14.7	34.5	9.1	56.5	61.2	58.3	47.7	36.1	19.3	1.8
Saskatoon	1.8	5.6	17.7	36.9	51.5	60.3	6 .4	62.4	51.4	38.9	22.6	6.9
Scott	2.1	5.6	16.8	36.5	49.9	58.0	63.3	60.7	50.0	37.8	22.1	5.7
Shaunavon	13.3	15.8	24.8	39.2	50.8	59.2	65.1	63.1	52.3	40.7	27.5	14.3
Swift Current	12.1	14.8	25.2	40.7	53.2	61.8	67.2	64.9	53.4	41.6	27.4	14.1
Waseca	1.8	6.5	17.1	36.5	50.1	57.4	62.4	59.8	49.1	36.9	21.0	5.5
Whitewood	2.7	6.7	18.4	36.4	49.9	58.7	63.5	60.9	50.7	38.	22.3	7.2
Yellow Grass	5.9	9.2	21.6	38.8	51.8	61.8	66.2	63.8	52.8	39.2	23.9	8.4
Mean	8.0	11.9	21.6	38.1	50.5	58.4	63.7	61.2	50.8	39.4	24.9	10.6
Standard deviation	6.51	5.63	4.13	2.10	2.29	2.94	2.87	2.72	2.17	1.92	3.13	4.81

Solution of the equations was effected by the inverse matrix method as systematized by Fisher (1, Sec. 29), the multipliers used in the computation of the unknowns and their standard errors, expressed as millionths, being:

	$c_{.1}$	$c_{.2}$	$c_{.3}$
$c_{1.}$	8.309592	−2.383167	0.929300
$c_{2.}$		1.638321	0.496299
$c_{3.}$			0.200340

The regression coefficients obtained, together with their respective standard errors, are assembled in Table III. With the exception of those for altitude in January and December, which are both less than twice their standard error, all are individually statistically significant.

TABLE III

PARTIAL REGRESSION COEFFICIENTS OF 18-YEAR AVERAGE (1917–1934) MONTHLY MEAN TEMPERATURE ON LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS

Month	Partial regression coefficient of temperature on		
	Latitude (°F. per 10′ N.)	Longitude (°F. per 10′ W.)	Altitude (°F. per 100 ft.)
January	−.56 ± .05	+.25 ± .02	−.13 ± .08
February	−.47 ± .05	+.23 ± .02	−.16 ± .08
March	−.50 ± .04	+.21 ± .02	−.36 ± .06
April	−.31 ± .03	+.13 ± .01	−.44 ± .05
May	−.24 ± .03	+.09 ± .02	−.51 ± .05
June	−.31 ± .04	+.06 ± .02	−.56 ± .06
July	−.36 ± .05	+.08 ± .02	−.56 ± .08
August	−.35 ± .04	+.07 ± .02	−.51 ± .06
September	−.29 ± .03	+.06 ± .01	−.42 ± .05
October	−.29 ± .03	+.10 ± .01	−.31 ± .05
November	−.37 ± .03	+.15 ± .02	−.22 ± .05
December	−.46 ± .05	+.19 ± .02	−.14 ± .08

The three series of coefficients present certain features of interest, for the relation of mean temperature to each of the three variables, latitude, longitude and altitude, changes in a marked but generally regular manner with the progress of the year. Each series has an independent seasonal trend, which is simplest in the case of altitude and most complex in the case of latitude. The coefficients for altitude increase steadily in magnitude from a minimum of −0.13° F. per 100 ft. in January to a maximum of −0.56° in June, then progressively decline to −0.14° in December. This is in agreement with the observation of Kincer (4) that the decrease in air temperature with altitude is greater in summer than in winter. On the other hand, the effect of longitude is most pronounced in winter. In January the mean temperature increases from east to west by an average amount of 0.25° F. for each 10′ longitude, after allowance is made for the effects of latitude and altitude. This effect diminishes rapidly in spring, however, and continues at a low level with little variation from May to September. The influence of latitude is likewise most pronounced in winter, being at its maximum in January when, after allowing for differences in longitude and altitude, there is an average decrease of 0.56° per 10′ N. In this case, however, the series of monthly coefficients exhibits two minima, one in the spring and the other in the autumn.

Fig. 2 shows the situation in graphical form. The points in this diagram represent the actual values of the regression coefficients listed in Table III, whilst the continuous curves show the course of harmonic equations fitted

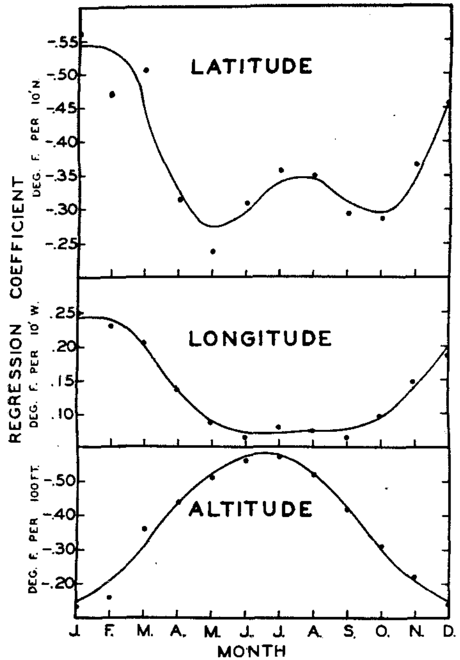

FIG. 2. *Seasonal trend of temperature gradients with latitude, longitude and altitude.*

to each series of 12 monthly coefficients by the method of Least Squares. The monthly regression coefficients of temperature on altitude follow very closely the simple harmonic equation

$$b_m = -0.36 + 0.22 \sin (\theta + 105°10')$$

where b_m is the regression coefficient for any specified month and $\theta = \frac{2\pi m}{12}$, m taking the values 0,1,2, ...,11 for the successive months of the year. A simple harmonic likewise accounts for most of the variation in the longitude coefficients, but in this case a statistically significant improvement results from the addition of a second harmonic term, giving

$$b'_m = 0.14 + 0.09 \sin (\theta + 76°10') + 0.02 \sin (2\theta + 57°55')$$

The latitude sequence is however represented only moderately well by

$$b''_m = -0.38 + 0.10 \sin (\theta + 261°50') + 0.08 \sin (2\theta + 241°40')$$

and the addition of the term in 3θ results in no significant improvement.

Table IV gives for each month the multiple correlation coefficient R between mean temperature and latitude, longitude and altitude, and the residual standard deviation s of the actual 18-year averages from the graduated values.

The correlation coefficients are all quite high, indicating that the linear regression equations have accounted for the major part of the recorded temperature differences between stations. When it is recalled that the residual standard deviation includes, as well as observational errors, any systematic non-linear deviations, any irregularities remaining in the station averages due to the small number of years included, and any effects arising from local topography or the exposure of individual stations, the results shown in Table IV cannot be regarded as wholly unsatisfactory. In some instances indeed it may well be that the graduated temperatures approximate more closely to the true values for the adjacent territory than do those recorded at the individual stations. It may be concluded therefore that Table III and Fig. 2 provide a fairly comprehensive numerical description of the temperature gradients characteristic of this region at different periods of the year.

TABLE IV

MULTIPLE CORRELATION COEFFICIENT (R) BETWEEN MEAN TEMPERATURE AND LATITUDE, LONGITUDE AND ALTITUDE, AND RESIDUAL STANDARD DEVIATION (s), BY MONTHS

Month	R	s, °F.
Jan.	0.96	1.9
Feb.	.96	1.7
Mar.	.95	1.3
Apr.	.87	1.1
May	.86	1.2
June	.91	1.3
July	.88	1.4
Aug.	.89	1.3
Sept.	.89	1.0
Oct.	.83	1.1
Nov.	.93	1.2
Dec.	.94	1.7

Results with Observations for Individual Years

The remarks of the preceding section apply of course to the 18-year averages of monthly mean temperature. In the means for any month of a single year, additional deviations from the regression equation may arise from two sources: (i) over the region as a whole, the temperature of the month in question may be above or below the climatological average; (ii) local irregularities, which tend to nullify each other in the averages of a number of years, will be more pronounced.

Annual differences of type (i), in so far as they affect all stations equally, need not increase the residual standard deviation, since they require only an adjustment in the constant term of the regression equation. Irregular local variations, on the other hand, will of course result in increased discrepancies between the observed and graduated values. In order to determine the extent of such effects in practice, the two extreme winter and summer months January and July were selected, and the total variance of the 18 × 43 annual monthly means for each was partitioned (1, Chap. VII) into components

due to (i) differences between the 18-year averages of the 43 individual stations; (ii) differences between the 43-station averages of the 18 individual years; and (iii) residual irregular local variation. These computations gave the mean square deviations shown in Table V.

TABLE V

ANALYSIS OF VARIANCE OF MONTHLY MEAN TEMPERATURE

Source of variation	Degrees of freedom	Mean square	
		January	July
Between station averages	42	785.38	147.72
Between year averages	17	2643.02	52.87
Residual	714	4.80	1.65

The January mean square between years is greatly in excess of the mean square residual, indicating a pronounced correlation between the annual variations in mean temperature of this month at the 43 stations. A similar tendency is to be noted in the results for July, but in this case the degree of intra-annual correlation indicated is appreciably lower.

TABLE VI

LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS, AND MONTHLY MEAN TEMPERATURES FOR THE YEAR 1935

Station	Lat. N. of 49°, min.	Long. W. of 103°, min.	Height above sea level, ft.	Monthly mean temp. 1935, °F.			
				Jan.	Apr.	July	Oct.
Beechy	110	266	2180	− 5.6	34.4	67.4	38.7
Biggar	183	299	2154	− 9.1	34.2	69.3	39.8
Caron	88	173	1841	− 2.5	37.7	68.8	38.4
Chaplin	88	220	2202	− 3.0	36.8	69.9	38.2
Davidson	136	179	2030	− 7.7	34.8	67.6	36.3
Dundurn	168	210	1737	− 8.3	35.2	69.0	38.7
Ft. Qu'Appelle	107	48	1600	− 4.6	36.8	67.0	37.9
Francis	67	50	1977	− 6.0	33.9	68.1	38.5
Gravelbourg	52	213	2297	− 3.8	36.3	68.9	38.4
Harris	164	273	1896	−10.4	33.9	68.1	41.9
Humboldt	192	129	1865	−11.6	31.9	68.0	38.3
Indian Head	88	40	1924	− 6.0	35.6	68.9	38.4
Lestock	137	57	2219	− 7.1	32.8	66.7	37.1
Lumsden	99	115	1620	− 5.0	37.7	68.8	39.8
Maskakee Springs	199	161	1787	−12.8	33.3	69.9	37.9
Moose Jaw	81	155	1860	− 1.1	37.7	70.4	39.9
Nokomis	150	120	1718	− 7.8	35.6	68.3	36.8
Outlook	148	245	1774	− 7.4	34.9	70.5	39.6
Pennant	92	314	2346	− 1.6	35.2	69.0	43.1
Qu'Appelle	91	56	2147	− 4.5	34.6	67.4	37.9
Regina	87	97	1884	− 5.8	36.9	68.7	38.5
Saskatoon	195	210	1600	−10.1	34.0	68.6	37.9
Saskatoon Univ.	188	218	1690	−10.0	34.5	69.2	38.6
Strasbourg	125	117	1799	− 6.8	35.7	67.3	39.3
Swift Current	80	285	2440	+ 2.3	36.4	68.4	40.4
Tugaske	111	196	1986	− 6.1	35.1	68.4	38.6
Yellow Grass	49	69	1899	− 2.6	35.3	69.2	38.8

When the residual variance in Table V, ascribable to local variation within years, was added to the residual variance of the 18-year station averages from the regression equation given in the preceding Table IV, standard deviations of 2.9° F. for January and 1.9° for July were obtained. These may be regarded as representative of the closeness of graduation to be expected on the average from the regression coefficients of Table III (after adjustment of the constant term), in individual years. The agreement thus indicated between the observed and graduated values can hardly be regarded as entirely satisfactory.

More recently, additional meteorological stations have been established, and records for the year 1935 are available for 27 stations situated within or just beyond the borders of the area bounded by the 50th and 52nd parallels and the 104th and 108th meridians. The mean temperatures for the months of January, April, July and October of that year at each of these points were accordingly tabulated, and are shown in Table VI, together with the latitude, longitude and altitude of the individual stations.

The Normal Equations to determine the regression coefficients of January mean temperature on latitude, longitude and altitude respectively were:

$$\begin{aligned} 54{,}264\, b_1 + 28{,}121\, b_2 - 116{,}241\, b_3 &= -3{,}496.1 \\ 28{,}121\, b_1 + 184{,}263\, b_2 + 180{,}373\, b_3 &= 168.5 \\ -116{,}241\, b_1 + 180{,}373\, b_2 + 1{,}432{,}561\, b_3 &= 10{,}371.2 \end{aligned}$$

whilst the corresponding values of the right-hand side for the other three months were:

−1093.2	25.9	−207.6
− 129.9	767.3	1721.5
− 707.6	−670.3	2146.2

Proceeding as before, the matrix of multipliers was found to be, in millionths

	$c_{.1}$	$c_{.2}$	$c_{.3}$
$c_{1.}$	29.83665	−7.89664	3.41527
$c_{2.}$		8.27989	−1.68326
$c_{3.}$			1.18711

giving the regression coefficients and standard errors shown in Table VII.

TABLE VII

PARTIAL REGRESSION COEFFICIENTS OF MEAN MONTHLY TEMPERATURE (1935) ON LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS

Month	Partial regression coefficient of temperature on		
	Latitude (°F. per 10′ N.)	Longitude (°F. per 10′ W.)	Altitude (°F. per 100 ft.)
January	−.70 ± .09	.12 ± .05	.01 ± .17
April	−.34 ± .05	.09 ± .03	−.44 ± .10
July	−.08 ± .05	.07 ± .03	−.20 ± .10
August	−.12 ± .06	.12 ± .03	−.11 ± .13

These coefficients are of course affected by any circumstances peculiar to the particular year and locality. Nevertheless on the whole their seasonal trend resembles that of the series previously computed for the 43 more widespread 18-year stations, although there are some discrepancies. The values of R, the coefficient of multiple correlation between mean temperature and the three co-ordinates of position, and of *s*, the residual standard deviation, were found to be as follows for the four months:

Month	*R*	*s*, °F.
January	0.90	1.6
April	.82	0.9
July	.52	0.9
October	.64	1.2

The correlation coefficients, although all statistically significant, are lower than those listed in Table IV, and the standard errors of the regression coefficients are relatively high (Table V). This is not surprising, since in the smaller area now under consideration irregular local effects would be expected to constitute an increased proportion of the total variance in temperature. On the other hand, the residual standard deviations for the four months are now appreciably lower than those deduced on page 25. Some addition to the number of stations is still desirable to reduce the standard deviation of the coefficients, the 27 listed in Table VI being distributed over an area of approximately 25,000 square miles. If this was effected however, it would seem that a reasonably accurate linear graduation of the air temperature gradients in this district, even in individual years, would be possible.

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THE EFFECT OF PHOSPHATE DEFICIENCIES ON INFECTION OF WHEAT BY *FUSARIUM CULMORUM*[1]

BY F. J. GREANEY[2]

Abstract

The influence of phosphate deficiencies on infection of wheat by *Fusarium culmorum* (W. G. Sm.) Sacc. was studied. Marquis wheat was grown in pot cultures of quartz sand with different types of manuring, including a fully manured control, and four series having deficiencies of phosphate. One-half of the pots were inoculated with *F. culmorum* and sown with inoculated seed, the remainder served as uninoculated controls. The plants were grown for 36 days. The experimental data were treated by the analysis of variance method.

Under the conditions of the experiment, deficiencies in phosphate did not significantly increase or decrease the susceptibility of wheat plants to root rot caused by *F. culmorum*. On the other hand, deficiencies in phosphate significantly reduced root development and total dry weight of the plants. The results suggest that the effect of phosphatic fertilizers is much more important on plant growth and yield than on the severity of infection by *F. culmorum*.

Introduction

For many years considerable attention has been paid to mineral nutrition as a factor affecting susceptibility to disease in the higher plants. This subject has been investigated in a number of fungus diseases of wheat, barley, and other small grain crops. In the literature, however, references to the influence of phosphorus on disease development are often of a casual nature, made in the course of investigations with other plant nutrients. It may be well, therefore, before reporting the results of the present study which deals particularly with phosphorus, to mention some of the results obtained by other workers on the effect of mineral nutrition in relation to disease in plants.

One of the earliest important contributions to this subject is by Spinks (16). According to him, heavy nitrogenous manuring increases the susceptibility of wheat and barley to their particular rusts and mildews, whereas potash acts in a contrary manner. He found that the effect of phosphorus upon susceptibility to disease in cereal plants is not always the same: in some cases it decreases susceptibility and in others it does not. The results of more recent work by Schaffnit and Volk (14), Gassner and Hassebrauk (4, 5), Eglits (2), and by some others, are essentially similar to those of Spinks.

The effect of fertilizers on the development of obligate parasites, such as the rusts of wheat, have been studied by several investigators. For instance, Biffen (1) and Voelcker (20) found that heavy applications of nitrogenous fertilizers increased the susceptibility of wheat to *Puccinia glumarum*. Spinks (16) and Voelcker (20) concluded that potassium and phosphorus salts increased the resistance of wheat to stripe rust but did not counteract the effect of large amounts of nitrogen. Vavilov (19) concluded that the apparent increased susceptibility of wheat to *Puccinia triticina*, when grown in soil

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Contribution No. 517 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
[2] *Plant Pathologist, Dominion Rust Research Laboratory, Winnipeg, Manitoba.*

fertilized with nitrogen, was due to increased development of leaf surface rather than to any change of real resistance. Stakman (17) and Stakman and Aamodt (18) found that excessive amounts of phosphorus salts had no direct effect on the resistance of wheat to *Puccinia graminis*, and concluded that any decrease in the amount of this rust due to phosphatic fertilizers was brought about by hastening the maturity of the host plant. Similar results with *Puccinia triticina* were reported by Greaney and Machacek (7). Most of these results indicate that nitrogen and potash influence the susceptibility of wheat to disease. The evidence, however, in regard to any clear-cut effect of phosphorus on resistance in wheat, even to such obligate parasites as the rust fungi, is not very convincing.

In regard to facultative parasites that attack crop plants, Neal (13), in 1927, observed that increasing the phosphate content of the soil did not decrease the tendency of cotton plants to become infected by *Fusarium vasinfectum* Atk. The work of McRae and Shaw (12) on the influence of manures on the wilt disease of Indian Pulse (*Cajanus indicus* Spreng.) indicated that manuring with superphosphate increased attack by *Fusarium vasinfectum*. They found that increasing the concentration of phosphate increases the growth of the fungus up to a certain point, after which higher concentrations check growth. McRae and Shaw (12) reported that, in sand culture experiments with cotton seedlings, Kulkarni and Mundkur found that high concentrations of potassium phosphate predispose plants to attack by *F. vasinfectum*.

The influence of mineral nutrition upon susceptibility to disease in plants has been studied for a number of fungi, with results that are in many cases indefinite. The records of these investigations do not give any clear proof that phosphorus directly affects the natural resistance to disease that wheat plants possess. It was therefore decided to grow numbers of wheat plants in sand culture under varying conditions of nutrition and to observe any differences in their susceptibility to the attacks of root rot caused by *Fusarium culmorum*. This paper presents the results of greenhouse studies to determine the effect of deficiencies in phosphate on infection of young wheat plants by *Fusarium culmorum* (W. G. Sm.) Sacc.

Experimental Methods

It was expected from the results of previous studies (9, 10) with pot cultures in quartz sand that the effects of deficiencies of phosphorus on the resistance of wheat seedlings to *Fusarium culmorum* would be of small magnitude, and would require data of a high order of precision for their establishment. The technique of growing plants in sand culture described by Gregory and Crowther (9), in their studies on the differential response in yield of barley varieties to manurial deficiencies, seemed well adapted for the investigation of the problem in question.

A preliminary sand-culture experiment with wheat grown under various phosphate manurings was carried out in 1932. Four different concentrations

of phosphorus, including a complete manure, two deficiency series, and a series receiving an excess of phosphorus, were employed. The complete manure provided nitrogen, potash, and phosphorus in the ratio of 3 : 2 : 1. In each deficiency series nitrogen and potash were provided in the same concentration as in the control series with complete manure, while phosphorus was lacking in one and present in $\frac{1}{5}$ of the complete amount in the other. The fourth manurial series received twice the amount of phosphorus supplied in the complete fertilizer. An unmanured control was included in the experiment. This preliminary test with about 50 inoculated and 50 uninoculated plants in each manurial series was made at two different times. The resulting data were treated by the analysis of variance method.

Evidence was thereby produced, which indicated that the pathogenicity of *Fusarium culmorum* to wheat might be influenced by the amount of phosphate given. The results of the test suggested a wider range of manuring, and led to a more efficient method of artificially inducing attacks with *F. culmorum* in sand culture.

In view of these results it was decided to repeat the experiment using a greater range of phosphate deficiencies. This enlarged greenhouse experiment was conducted at Winnipeg in 1933, repeated at Winnipeg in 1934, at Rothamsted Experimental Station, Harpenden, England, in 1935, and again at Winnipeg in 1936. During the course of each trial, uniform conditions of light, moisture, and temperature prevailed. At Winnipeg the temperature range was from 22° C. to 25° C.; while the range at Rothamsted was from 20° C. to 26° C. To minimize place effect the pots of each trial were completely randomized on a large bench in the centre of the greenhouse.

The complete experiment involved 60 pots. The pots were of white glazed earthenware holding 15 pounds of dry white quartz sand. Before use, the sand was washed three times in tap water, rinsed twice in distilled water, and sterilized under steam pressure. The manures were added in solution and the pots of sand brought up to uniform moisture condition by the addition of distilled water. The pots were then planted with seed of Marquis wheat. The water content was maintained at a uniform level by adding distilled water at two-day intervals to bring the pots up to their original weight. Waterlogging in the pots was avoided by allowing percolation. The percolated water was collected and subsequently returned to the pots. The manuring scheme employed in the experiment is given in Table I.

TABLE I
MANURIAL TREATMENTS
(Amounts in grams per pot)

Series / Constituent	Complete manure	Phosphate-deficient series				Unmanured control
	A	B	C	D	E	F
N	0.75	0.75	0.75	0.75	0.75	0.0
K_2O	0.50	0.50	0.50	0.50	0.50	0.0
P_2O_5	0.25	0.05	0.025	0.0125	0.0	0.0

The complete manure provided nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5) in the ratio of 3 : 2 : 1. In each deficiency series the two constituents not in deficiency were supplied in the same concentration as in the series with complete manure, while phosphorus was present in 0, $\frac{1}{50}$, $\frac{1}{10}$, and $\frac{1}{2}$ of the complete amount. This gave a range of grades of deficiency in phosphorus in the presence of adequate amounts of the other constituents. Nitrogen was added as $NaNO_3$, phosphate as Na_2HPO_4, $12H_2O$, and the potash as K_2SO_4. Calcium (0.19 gm. $CaCl_2$), magnesium (0.63 gm. Mg_2SO_4, 7 H_2O), and a trace of iron ($FeCl_3$) were added to each pot. The solutions were brought to an initial pH of 5.8 by adding sulphuric acid. Resulting variations in the amount of sodium were corrected by adding Na_2SO_4, 10 H_2O. On the basis of a 19% water content of the sand, the concentration of sodium was far below that necessary to produce the slightest toxic effect (11). The only other ion that varied was SO_4. It has been shown to have a very small effect on plant growth in sand culture (8).

The seed used was Marquis wheat, selected by hand for uniformity in size and color. It was surface-sterilized by rinsing in 75% ethyl alcohol, immersing for three minutes in 0.1% $HgCl_2$ solution, rinsing in alcohol, and then washing in sterile water. Seed treated in this manner gave a germination of 99% in blotting-paper and sand tests.

Before sowing, one-half of the surface-sterilized seed was inoculated by dipping it in a suspension of spores and mycelial fragments of *Fusarium culmorum*. The control seed was dipped in sterile water. Both lots were sown immediately after treatment.

The particular fungus used in this investigation was a strain of *Fusarium culmorum* (W. G. Sm.) Sacc. that was originally isolated in 1930 from a rotted crown of Marquis wheat. Previous tests (6) had demonstrated that this strain was definitely pathogenic to wheat.

In each manurial series there were 10 pots, five sown with inoculated seed and five with uninoculated seed to serve as control. Twenty seeds were sown in each pot. By means of a small sterile glass rod, holes were made to a uniform depth of one inch below the surface of the sand. A single seed was placed in each hole. About 5 cc. of a water suspension of spores of *F. culmorum* was poured over each inoculated seed before it was finally covered with sand. The same quantity of sterile water was poured over each uninoculated seed. The seeds were covered by lightly packing the surface layer of sand.

Germination was usually complete 10 days after sowing, at which time the plants in each pot were reduced to a uniform number. In three of the four trials, the total number of post-emergence plants per inoculated and uninoculated series of each manurial type was about 95.

At the end of the experimental period (36 days), non-emerged plants as well as the young seedlings were lifted from the pots, washed free of sand, examined individually, and the extent of injury due to pre-emergence blight, seedling blight, or root rot was recorded. The classes and numerical ratings

used to record the intensity of disease infection on individual plants, and the method of computing the disease rating that was used to express the extent of the disease on the plants in each series of pots, are given in Table II.

TABLE II

CLASSES, NUMERICAL RATINGS, AND DISEASE RATING USED TO RECORD THE DEGREE OF INFECTION BY *Fusarium culmorum* ON WHEAT PLANTS

Class	Degree of infection on individual plants	Numerical rating
1	No infection	0
2	Small, scattered necrotic lesions on sheath, sub-crown internode, or roots	1
3	Distinct lesions on basal parts, particularly on sub-crown internode and roots	2
4	Large necrotic lesions on crown, sub-crown internode and roots, with loss of plant vigor	3
5	Severe rotting of basal parts; plants chlorotic, often stunted or wilted; some culms dead	4
6	Plant destroyed after germination but before emergence. Dead plant	5

$$\textit{Disease rating} = \frac{\textit{Sum of numerical ratings} \times 100}{\textit{Number of plants at 36 day.} \times 5}$$

After the disease data had been secured, the plants were air-dried and the total dry weight per inoculated and uninoculated set of each manurial series was recorded.

Plant emergence, disease, and yield data were analyzed according to the procedure described by Fisher (3) as the analysis of variance. To estimate the odds of significance, however, the direct ratio of the variances, the F value of Snedecor (15), was used.

Experimental Results

The results of the preliminary experiment in 1932 (Table III) show the effects of different manurings with phosphate on infection by *Fusarium culmorum* and on the growth of young wheat plants. To economize space, the complete analysis of variance for disease-infection rating and total dry weight of plants is not given. The results of these analyses, however, established that, for the amount of disease and total dry weight, the effects of seed inoculation with *F. culmorum* were very great. In this experiment as a whole, the differences in disease infection for manurial treatments were not statistically significant; whereas the differences observed in plant growth, due to different phosphate manures, were very significant.

The evidence presented in Table III shows that there is a tendency, although the differences are not significant statistically, for decreasing concentrations of phosphate to increase infection by *F. culmorum.* Under the conditions of the experiment, an excess of phosphate still further increased infection by this fungus. Decreasing concentrations of phosphate decreased root development and general plant growth.

TABLE III

INFLUENCE OF DIFFERENT PHOSPHATE MANURES ON INFECTION OF WHEAT BY *Fusarium culmorum*, AND ON TOTAL DRY WEIGHT OF PLANTS

(Average results of two preliminary trials)

Manurial series	Manuring scheme, gm. per pot			Degree of infection (disease rating)		Total dry weight,* gm.	
				Seed treatment			
	N	K_2O	P_2O_5	Inoculated	Control	Inoculated	Control
Complete	0.75	0.5	0.25	41.8	8.0	6.4	8.2
P-deficient	0.75	0.5	0.05	47.6	7.2	6.1	7.8
P-deficient	0.75	0.5	0.0	60.2	8.4	4.2	6.6
No fertilizer	0.0	0.0	0.0	60.1	14.2	3.6	5.4
P-excess	0.75	0.5	0.5	64.7	5.7	3.6	6.2
Mean of seed treatments				54.9	8.7	4.8	6.8
S.E. of means of seed treatments				±2.51		±0.22	

*Standard error of manurial treatments for total dry weight = ±0.16.

The complete data of the enlarged experiment with six manurial series— a fully manured control, four deficiency series, and a complete starvation series— are given in Table IV. This table presents the data recorded on percentage of plants emerged, percentage of plants diseased, intensity of infection by *F. culmorum*, and total dry weight of plants per series of five pots with inoculated and with uninoculated seed. The figures are the means of each series.

The data of the four trials were treated by the analysis of variance method (Table V). The significance of the results were assessed by the F test in which the variance due to any known cause is compared directly with the variance due to error.

The results of the analyses in Table V show that the effects of seed inoculation with *F. culmorum* are very great, as would be expected. In every case the variances for seed treatments greatly exceed the error variances. Thus the results establish the efficiency of the method used to induce positive attacks with *F. culmorum*.

The efficiency of the method being established, a more detailed examination was made of the effects of deficiencies of phosphate on disease development and plant growth. This examination of the data shows that, owing to the four stages of deficiency of phosphate employed, the differences in number of plants emerged after 10 days, and in number of plants diseased, and degree of infection by *F. culmorum* after 36 days, are not significant. In Table V the variance for manurial treatments in the case of plants emerged, plants diseased, and disease rating, is not as great as, or significantly greater than, the variance due to error. This establishes, therefore, that the differences observed between manurial treatments might easily have arisen from chance alone.

TABLE IV

PERCENTAGE OF PLANTS EMERGED, PERCENTAGE OF PLANTS DISEASED, DEGREE OF INFECTION BY *Fusarium culmorum*, AND TOTAL DRY WEIGHT OF WHEAT PLANTS IN FOUR TRIALS WITH DIFFERENT PHOSPHATE MANURES

Trial	Seed treatment	Complete fertilizer (N.P.K.)	Phosphate-deficient series				Unmanured control
			$\frac{1}{2}$ P	$\frac{1}{10}$ P	$\frac{1}{20}$ P	0 P	
Percentage of plants emerged							
Winnipeg, 1933	Inoculated	100	97	99	99	100	100
	Uninoculated	100	99	100	99	98	100
Winnipeg, 1934	Inoculated	99	95	96	100	97	93
	Uninoculated	96	100	100	100	98	100
Rothamsted, 1935	Inoculated	100	98	98	100	98	98
	Uninoculated	100	100	100	100	100	100
Winnipeg, 1936	Inoculated	65	75	85	81	70	76
	Uninoculated	94	97	95	95	94	94
Percentage of plants diseased							
Winnipeg, 1933	Inoculated	100	100	100	100	100	100
	Uninoculated	20	28	29	22	23	43
Winnipeg, 1934	Inoculated	100	100	100	98	100	100
	Uninoculated	39	30	34	25	11	29
Rothamsted, 1935	Inoculated	67	78	82	80	93	85
	Uninoculated	13	12	13	8	12	18
Winnipeg, 1936	Inoculated	100	100	100	100	100	100
	Uninoculated	13	16	21	17	43	57
Degree of infection (Disease rating)							
Winnipeg, 1933	Inoculated	62.5	68.0	70.2	70.3	54.9	53.2
	Uninoculated	4.8	6.6	6.2	6.8	6.2	10.0
Winnipeg, 1934	Inoculated	50.7	50.7	41.3	33.4	43.4	34.6
	Uninoculated	12.0	8.4	9.0	5.0	5.6	6.2
Rothamsted, 1935	Inoculated	13.3	15.6	16.9	20.0	25.1	24.4
	Uninoculated	3.4	3.0	3.3	1.7	2.3	3.7
Winnipeg, 1936	Inoculated	47.6	62.7	66.4	63.6	62.6	72.3
	Uninoculated	3.0	3.6	4.4	3.4	10.3	24.8
Dry weight of plants (grams)							
Winnipeg, 1933	Inoculated	23.4	20.3	22.5	16.5	19.0	11.2
	Uninoculated	24.5	24.3	27.1	22.7	19.5	14.8
Winnipeg, 1934	Inoculated	15.4	14.5	17.8	14.5	11.0	10.0
	Uninoculated	17.6	18.7	17.9	19.3	15.2	12.1
Rothamsted, 1935	Inoculated	14.0	15.2	14.0	14.8	13.6	8.8
	Uninoculated	17.8	16.2	14.4	15.4	14.3	9.4
Winnipeg, 1936	Inoculated	8.5	7.9	9.1	8.5	8.5	3.5
	Uninoculated	14.9	13.8	14.4	14.3	14.2	6.2

It is clear from Table V, however, that a significant difference for manurial treatments is obtained in the case of total dry weight of plants. The F value greatly exceeds the 5% point. This result indicates that a high degree of significance can be attached to the effect of phosphate deficiencies on plant

TABLE V

COMPLETE ANALYSIS OF VARIANCE FOR PERCENTAGE OF PLANTS EMERGED, PERCENTAGE OF PLANTS DISEASED, DISEASE RATING, AND TOTAL DRY WEIGHT OF PLANTS

Variance due to	Degrees of freedom	Sum of squares	Mean square	F	5% point
	Percentage of plants emerged				
Experiments	3	1,711.73	570.57		
Fertilizers	5	46.73	9.35	—	—
Seed treatments	1	408.33	408.33	13.31	4.12
Fertilizers × seed treatments	5	26.17	5.23		
Error	33	1,012.27	30.67		
Total	47	3,205.23			
	Percentage of plants diseased				
Experiments	3	2,944.23	981.41		
Fertilizers	5	1,081.94	216.38	2.04	2.48
Seed treatments	1	58,590.18	58,590.18	554.20	4.12
Fertilizers × seed treatments	5	825.94	165.18	1.56	2.48
Error	33	3,489.03	105.72		
Total	47	66,931.32			
	Disease rating				
Experiments	3	4,697.48	1,565.82		
Fertilizers	5	80.81	16.16	—	—
Seed treatments	1	19,602.08	19,602.08	146.10	4.12
Fertilizers × seed treatments	5	120.46	24.09		
Error	33	4,427.60	134.17		
Total	47	28,928.43			
	Total dry weight				
Experiments	3	637.49	212.49		
Fertilizers	5	333.44	66.69	20.90	2.48
Seed treatments	1	121.92	121.92	38.22	4.12
Fertilizers × seed treatments	5	6.25	1.25		
Error	33	105.20	3.19		
Total	47	1,204.30			

growth as expressed by total dry weight. In all cases the interactions in the experiment are not significant.

The results of the experiment, with the standard errors associated with the various factors studied, are summarized in Table VI.

TABLE VI

INFLUENCE OF DEFICIENCIES OF PHOSPHATE ON THE PATHOGENICITY OF *Fusarium culmorum* TO WHEAT SEEDLINGS, AND ON PLANT GROWTH

(Average of four trials)

Manurial series	Manuring scheme, gm. per pot			Percentage of plants emerged (after 10 days)		Percentage of plants diseased (after 36 days)	
				Seed treatment			
	N	K_2O	P_2O_5	Inoculated	Control	Inoculated	Control
Complete	0.75	0.50	0.25	91.0	97.5	91.8	21.2
P-deficient	0.75	0.50	0.05	91.2	99.0	94.5	21.5
P-deficient	0.75	0.50	0.025	94.5	98.8	95.5	24.2
P-deficient	0.75	0.50	0.0125	95.0	98.5	94.5	18.0
P-deficient	0.75	0.50	0.0	91.2	97.5	98.3	22.2
Control	0.0	0.0	0.0	91.8	98.5	96.2	44.2
Mean of seed treatments				92.4	98.3	95.1	26.9
S.E. of means of seed treatments				±1.13		±2.10	
				Degree of infection (Disease rating)		Total dry weight of plants, (gm)*	
Complete	0.75	0.50	0.25	43.5	5.8	15.3	18.7
P-deficient	0.75	0.50	0.05	49.2	5.4	14.5	18.2
P-deficient	0.75	0.50	0.025	48.7	5.7	15.8	18.4
P-deficient	0.75	0.50	0.0125	46.8	4.2	13.6	17.9
P-deficient	0.75	0.50	0.0	46.5	6.1	13.0	15.8
Control	0.0	0.0	0.0	46.1	11.2	8.4	10.6
Mean of seed treatments				46.8	6.4	13.4	16.6
S.E. of means of seed treatments				±2.36		±0.36	

**Standard error of manurial treatment for total dry weight* = ±0.63.

The evidence presented in Table VI establishes the fact that positive attacks were induced by inoculating the seed and sand with spores and mycelial fragments of *F. culmorum*. The inoculation reduced plant emergence 5.9%, increased the number of diseased plants 68.2%, and raised the disease rating from 6.4 to 46.8. In each manurial series plants from inoculated seed were much smaller than plants from uninoculated seed (Fig. 1, A). The difference between the mean total dry weight of inoculated plants and uninoculated plants is 3.2 gm. This value exceeds three times the standard error of the means for total dry weight of plants. It can therefore be assumed that the difference of 3.2 gm. indicates a real difference between inoculated and uninoculated plants.

Under the conditions of the experiment, increasing deficiencies of phosphate did not render young wheat plants more susceptible to *Fusarium culmorum*. On the other hand, evidence was obtained which indicated that high concentrations of phosphate tend to predispose plants to attack. This result is

similar to that found by Neal (13) and by McRae and Shaw (12) with *Fusarium vasinfectum*. The results herein reported show that deficiencies in phosphate reduced root development and decreased the vigor of wheat seedlings (Fig. 1,B).

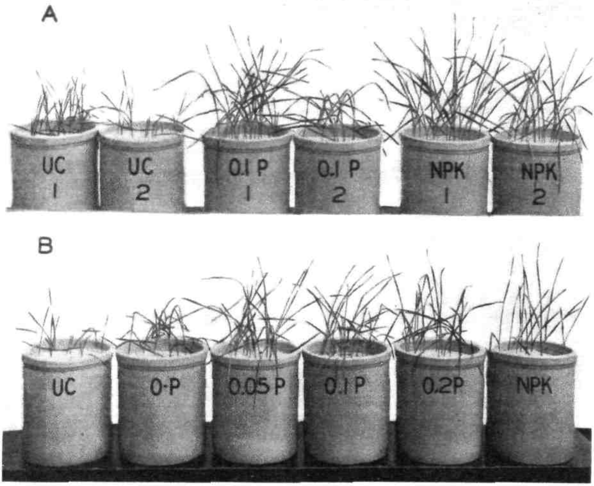

FIG. 1. *Influence of phosphate deficiencies on infection of wheat by Fusarium culmorum, and on plant growth. A. Effect of inoculation with F. culmorum. UC, unmanured control; NPK, complete manure; O.1-P, received $\frac{1}{10}$th the amount of phosphorus provided in NPK. Pot 1, uninoculated control; Pot 2, seed and sand inoculated with F. culmorum. B. Effect of deficiencies in phosphate on the growth of Marquis wheat at 15 days. UC, unmanured control; NPK, complete manure. In other series nitrogen (N) and potash (K) supplied in the same concentration as in the complete manure, while phosphorus (P) was given in the ratio of 0.0, 0.05, 0.1, and 0.2 to the complete amount.*

In the present experiments, the fungus *F. culmorum* attacked the roots of young wheat plants with equal vigor, regardless of the concentration of phosphoric acid employed; no statistically significant differences in their resistance to *F. culmorum* were established. The results gave no clear proof that phosphate directly affects the natural resistance of wheat plants to a root disease caused by *F. culmorum*, or affected in any way the parasitic vigor of the pathogen.

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THE ORIGIN OF ABNORMAL RUST CHARACTERISTICS THROUGH THE INBREEDING OF PHYSIOLOGIC RACES OF *PUCCINIA GRAMINIS TRITICI*[1]

By Thorvaldur Johnson[2] and Margaret Newton[3]

Abstract

The inbreeding of physiologic races of *Puccinia graminis Tritici* by means of the selfing of certain selected strains for several successive generations has given rise to rust strains with various abnormal characteristics manifested not only in the uredial and telial stages but also in the pycnial and aecial stages. These abnormalities include: (i) Abnormal uredial color—grayish-brown, orange, white. (ii) A decrease in the vigor of sporulation, that is, a tendency to form uredia that fail to rupture the epidermis of the wheat plant. (iii) A decrease of pathogenic vigor in certain strains, as shown, for example, by a tendency towards an "x" type of infection in cultures descending from strains that appeared homozygous for the more vigorous "4" type of infection. Certain strains originating through selfing have also shown a greater sensitivity to high temperatures than strains collected in the field. Wheat varieties susceptible to such strains at ordinary greenhouse temperatures develop resistance towards them at temperatures above 80° F. whereas their reaction to cultures collected in the field remains almost unaffected. (iv) Loss of ability to produce aecia on the barberry. (v) The development of uredia and telia on the barberry by some strains that have, partially or entirely, lost the capacity to produce aecia.

The development of abnormal strains of rust is not an inevitable consequence of inbreeding, as many inbred strains show no abnormal characteristics. It is suggested that the abnormal characteristics are, in most cases, the result of recessive mutations that have taken place in the past history of the rust, the part played by the selfing being that of segregating and recombining the mutant factors in a homozygous state under which condition their effects are manifested in various types of abnormalities.

Introduction

For several years the writers have attempted to study the inheritance of pathogenicity and other characteristics of physiologic races of *Puccinia graminis Tritici* Erikss. & Henn. This study has been carried out by means of crosses or matings between the races, which, in turn, were followed by progeny studies involving the selfing of certain selected strains for several successive generations. Certain aspects of this work have been dealt with in previous papers (7, 10, 12, 13). In the course of these selfing studies, rust strains with various abnormal characteristics have appeared. The purpose of the present paper is to describe these abnormalities and, as far as possible, to explain their origin in the light of our present knowledge of the rust organism.

Before these abnormalities are considered it may be advisable to describe briefly the manner in which the selfing of physiologic races is carried out. In selfing a physiologic race, the race is first brought into pure culture by starting from a single urediospore, a single uredium, or a single aecial cup (aecium). The pure culture is then used to inoculate adult wheat plants on which teliospores subsequently develop. In order to ensure that no contamination by

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Contribution No. 516 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
[2] *Plant Pathologist, Dominion Rust Research Laboratory, Winnipeg, Canada.*
[3] *Senior Plant Pathologist.*

other races has taken place during this process it is essential to ascertain the purity of the race just prior to the formation of teliospores on the adult plants. When the teliospores have passed through their period of dormancy and have been induced to germinate they are used to inoculate barberries. After the appearance of the pycnial stage the pycniospore-containing nectar of numerous pustules is carefully intermixed, precautions being taken to prevent any accidental contamination by the pycnial nectar of other races. The resulting aecia are then used to inoculate wheat seedlings. With the appearance of the uredial stage on the wheat seedlings, the physiologic race has passed through its whole life cycle, for example, from an F_1 to an F_2 generation, without any intercrossing with other races. The physiologic race has therefore been selfed. Repeated selfings, generation after generation in the same line, amount to a process of inbreeding and may eventually result in genetically pure lines of the rust. Inbreeding for several successive generations, such as has been carried out in the laboratory, presumably occurs rarely, if ever, in nature.

One of the difficulties encountered in selfing studies is that of securing a population truly representative of the generation (F_2, F_3, etc.) that is to be studied—a matter which has already been discussed in some detail by the present writers (10, pp. 38–39). The method that has been adopted comprises a random selection of aecia, each aecium being used to initiate a uredial culture. As such cultures commonly contain but one physiologic race each, this method ensures that isolated phenotypes are being studied rather than mixtures of phenotypes, as frequently happens when all the aecia of a pustule are used to establish a culture.

The Effect of Inbreeding on the Uredial Stage

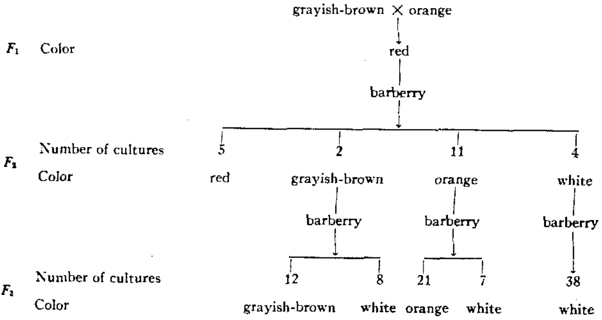
The Occurrence of Strains of Abnormal Uredial Color

Strains of abnormal uredial color are very rarely found in field collections of stem rust. Although field collections from various parts of Canada have been studied annually for 18 years, only one authentic record exists of the collection of uredia of abnormal color. As these uredia, which were grayish-brown in color, were collected on grasses in close proximity to an infected barberry plant, it is probable that they originated from infections caused by aeciospores. Although such strains are rarely found in nature, they have been known to originate in the greenhouse as mutants from races of normal uredial color. One such mutant with orange uredia has been reported by Newton and Johnson (9), and another with uredia designated as Mars Yellow has been described by Waterhouse (16). Nevertheless, cultures of stem rust, while they remain in the uredial stage, rarely give rise to such strains. When, however, barberries are infected with sporidia from teliospores collected in nature, some of the resulting aecia occasionally produce races of abnormal uredial color. The first record of a color variant originating in this manner—Race 36 (grayish-brown) of wheat stem rust—was described by Newton and Johnson (9). The origin, in a similar manner, of uredia of abnormal color has

also been recorded in other varieties of stem rust. Newton and Johnson (10, p. 34) obtained grayish-brown uredia from aecia on a barberry plant infected with a field collection of teliospores of *Puccinia graminis Secalis* Erikss. and Henn. In oat stem rust, color variants with orange uredia have been obtained by Gordon (4, p. 189) from a barberry that had been artificially inoculated with teliospores gathered in the field.

Although, in the above-mentioned examples, the teliospores may have represented a mixture of races, there is abundant evidence that, when pure cultures of physiologic races of normal spore color are selfed, they may produce color variants. Grayish-brown variants have occurred in the selfing of pure cultures of races of wheat stem rust collected in the field, and orange variants have occurred in the selfing of a pure culture of Race 6 of oat stem rust.

It is, however, from a study of crosses between color variants of physiologic races, rather than from selfing studies with field cultures, that some light has been thrown on the inheritance of this type of abnormality. Since the discovery of the first color aberrations (9), a number of crosses have been made between races of *P. graminis Tritici* that differed from each other in uredial color. A cross between a strain with orange uredia and one possessing grayish-brown uredia produced an F_1 hybrid race with normal (red) uredia (13). A selfing of the hybrid race produced an F_2 generation composed of cultures with red, grayish-brown, orange, and white uredia. Other crosses between orange and grayish-brown races have produced similar results. Selfings of F_2 and F_3 cultures have shown that the red cultures fall, genotypically, into four classes. Some are homozygous for red spore color; some produce red and orange progeny; others produce red and grayish-brown progeny; while still others produce all four color types; namely, red, grayish-brown, orange and



TEXT-FIG. 1. *Part of a progeny study of a cross between a race with grayish-brown uredia and a race with orange uredia, showing color segregation resulting from the selfing of red, grayish-brown, orange and white races. Of the* F_2 *cultures, only one grayish-brown, one orange, and one white culture were selfed.*

white. The grayish-brown strains when selfed fall into two classes. Some are homozygous for grayish-brown color; others produce grayish-brown and white strains. Similarly, the orange strains are either homozygous for orange color or produce orange and white cultures. The white strains, when selfed, produce only cultures with white uredia. Text-fig. 1 shows a typical progeny study of a cross between grayish-brown and orange races.

From a consideration of the behavior of the color variants in crosses and selfing studies it is clear that red spore color is dominant over grayish-brown, orange, and white, and that grayish-brown or orange spore color is dominant over white.

As color variants originate not infrequently from the selfing of physiologic races of normal color collected in nature, it is obvious that the genetic factors responsible for this difference in color are inherent in certain strains of the rust. Being recessive, these factors would have no visible effect while they are present in a heterozygous condition. When these factors are brought into a homozygous condition by selfing, their effect becomes apparent in uredial color deviating from the normal. A detailed scheme of the inheritance of urediospore color has been suggested by Johnson, Newton, and Brown (7).

The extreme rarity of strains of abnormal color in nature would suggest that such strains have a lower survival value than strains of normal color. At present, the reasons for this phenomenon can only be surmised. The spore-pigmentation characteristic of stem rust is possibly an ideal protective agent against the ultra-violet light produced by the sun as is, indeed, suggested by the work of Dillon Weston (2), who demonstrated that the urediospores of orange and white strains were more readily destroyed by artificially produced ultra-violet light than those of red or grayish-brown strains. Furthermore, it is possible that the urediospores of color variants are less viable than those of normal strains as has, in fact, been demonstrated by Newton and Johnson (9) for the first two color variants described by them. The germinability of the urediospores of these variants was considerably lower than that of rust spores of normal color. There is also reason to suppose that the urediospores of color variants are viable for a shorter period than spores of normal strains. This conclusion has been derived from experience with the storage of urediospores at about 10° C. and 50% relative humidity, under which conditions it is frequently found that, after a certain period of storage, the spores of the color variants fail to produce infection on wheat seedlings, whereas spores of normal color are still capable of causing infection.

The Occurrence of Strains Showing a Decrease in the Vigor of Sporulation

Another abnormal characteristic that has occasionally been noted in inbred races, but not in races collected in nature, is the tendency to form uredia which fail, partially or entirely, to rupture the epidermis of the wheat plant. Strains of this type have, for the sake of convenience, been designated as "subepidermal" (Plate I, Fig. 1). Although never collected in nature they have

originated from the selfing of two physiologic races collected in the field, namely, Race 21 and Race 36. In a selfing of the former race, one culture of a progeny of 35 cultures showed this characteristic; in a selfing of the latter race two cultures of a progeny of 23 cultures were subepidermal. It is evident, therefore, that the hereditary factors governing this condition are occasionally present in physiologic races collected in nature.

Subepidermal strains occur, however, more frequently in the selfing of cultures derived from crosses between physiologic races. In the F_2 generation of a cross between Races 36 (grayish-brown) and 9 (red), 7 out of 199 cultures were more or less subepidermal. Two of the F_2 cultures that formed pustules in a normal manner produced, when selfed, an F_3 progeny containing supepidermal strains. Of one of these, Race 36 (grayish-brown), 45 F_3 cultures were studied and classified as follows, according to the ability of the pustules to rupture the epidermis.

Normal pustule development	2
Very slightly subepidermal	5
Slightly subepidermal	7
Partially subepidermal	21
Entirely subepidermal	10

Although most commonly observed in races of wheat stem rust, subepidermal strains have also been noted, on one occasion, in selfing studies with physiologic races of oat stem rust. In the selfing of Race 6 (orange), 3 subepidermal cultures occurred in a progeny composed of 36 cultures. Race 6 (orange) itself was derived from the selfing of Race 7 (orange) which, in turn, originated from aecia on a barberry infected by teliospores collected in the field. If the teliospores from the field are considered as the F_1 generation, the subepidermal condition first became apparent in the F_4 generation, that is, after three consecutive selfings.

It is clear from the instances already cited that the occurrence of subepidermal strains is associated with the inbreeding of physiologic races. The formation of such strains does not, however, appear to be a necessary consequence of inbreeding. Many strains of wheat stem rust that have been selfed for several successive generations show no decrease in their vigor of sporulation. The explanation is more likely to be found in the genotypical characteristics of certain strains, the selfing of which tends to segregate and bring together the factors responsible for the subepidermal condition. Neither is the subepidermal condition necessarily associated with abnormal urediospore color, for many subepidermal strains have urediospores of normal color.

As no subepidermal strains have yet been selfed, nothing can be said at present concerning their breeding behavior.

The Occurrence of Strains Showing a Weakening of the Pathogenicity of the Rust

It might be expected that further inbreeding would have no effect on the pathogenicity in physiologic races that have already proved homozygous

for pathogenicity, as judged by the infection types produced on the differential wheat varieties. That is to say, the progeny of further selfings should consist of cultures pathogenically identical with each other and with the parent culture. Although this expectation has been borne out in the majority of selfing studies with homozygous races there have, nevertheless, been exceptions in which unexpected results were obtained. The exceptions thus far noted have represented a change in one direction, namely, towards a lower level of pathogenic vigor, such as a change from a "4" type of infection to a "3"± or an "x" type.

One of the selfing studies carried out with Race 9 furnishes an example of such a degeneration of pathogenic vigor. Race 9, when originally selfed, proved homozygous for pathogenicity. The 40 F_2 cultures studied were alike and produced a "4" type of infection on the varieties Mindum, Spelmar, and Vernal (12). Other selfings on a smaller scale confirmed the conclusion that the original culture was homozygous for pathogenicity. One of the F_2 cultures derived from the original selfing of this race was retained and kept in culture in the greenhouse for several years, with intervals of storage in a refrigerator at 10° C. and a relative humidity of 50%. Four years after the original selfing, adult wheat plants were inoculated with this culture with the object of producing teliospores for a selfing study. The usual checking of the purity of the race prior to teliospore formation showed no trace of contamination. When, however, this F_2 culture was selfed it produced, instead of a progeny composed entirely of Race 9, an F_3 generation composed of 15 cultures of Race 9, 5 cultures of Race 17, 19 cultures of Race 29, 9 cultures of Race 85 and 11 cultures of Race 149, a hitherto undescribed race. Other peculiarities were noted such as the fact that one of the 15 cultures of Race 9 produced uredia which were almost entirely subepidermal while two others produced uredia described as Mars Yellow in color.

A comparison of the infection types produced by the five physiologic races that arose from this selfing will show that the deviation from Race 9 is in every instance a degradation of infection type from a "4" type to an "x" type, and even to a "1" type in the case of the variety Vernal (Table I). It should be noted also, that the "x" type of infection produced by different cultures, identified as the same physiologic race, varied considerably in vigor. Thus two cultures, both identified as Race 149, showed a marked difference in the vigor of the "x" type of infection produced on Mindum and Spelmar (Plate I, Fig. 2). The classification of the cultures into five physiologic races does not, therefore, give an adequate idea of the variation in pathogenicity occurring in the progeny of this selfing. Nevertheless the variation in pathogenicity is not of the kind usually obtained in the selfing of a heterozygous race. Although the progeny was divided into five physiologic races, the pathogenic differences of these are slight except on the variety Vernal that clearly differentiates Races 17 and 29 from the other three races. With this exception, the races derived from this selfing bear a close resemblance to the parent Race 9. If the pathogenic differences in the progeny were due to an

undetected contamination by some other race, a more distinct variation in pathogenicity would have been expected.

It is difficult to account for the above-mentioned facts without having recourse to the supposition that a mutation or more probably a series of mutations had occurred in this race in the interval between the original and the present selfing. As this race gave rise to the first known mutant in stem rust, a color mutant also described as Race 9 (9), it is possible that mutations recur in this race from time to time. If this view is accepted it is clear that the degradation of pathogenicity and the development of other abnormal characteristics are results of inbreeding only in so far as inbreeding tends to segregate the hereditary factors changed by mutation. As these characteristics were not expressed in the parent race, the factors governing them are probably recessive.

Evidence of the decrease of pathogenic vigor in inbred strains has recently been secured in a different manner. Experiments on the effect of high temperatures on the rust development of physiologic races of wheat stem rust have shown that inbred strains are more sensitive to high temperatures than physiologic races collected in nature (6). Most inbred races failed to produce normal types of infection on susceptible wheat varieties when the mean daily greenhouse temperature exceeded 80° F. At higher temperatures the ordinary "4" type of infection of these races was replaced by a "3" type or an "x" type of infection or even by necrotic flecks (Plate I, Fig. 3). Races collected in the field were less affected by temperature. Of five such races that were tested, four showed little or no displacement of their ordinary infection type, when subjected to a mean daily temperature of 95°–99° F., whereas one race tended to produce an "x" type of infection at mean daily temperatures of 85°–89° F. and failed entirely to develop at higher temperatures. It is evident that differences in response to temperature exist among races collected in nature but, even after allowance is made for such differences it is clear that inbred races are less tolerant of high temperatures than those collected in the field.

If inbreeding occurs to any extent in nature, it is likely that strains similar to those produced in the laboratory would appear. If they do appear it is, however, probable that they have a low survival value and are consequently seldom collected.

TABLE I

INFECTION TYPES PRODUCED BY THE SELFED F_2 CULTURE OF RACE 9 AND BY THE FIVE PHYSIOLOGIC RACES OCCURRING IN THE F_3 PROGENY

Physiologic race	Little Club	Marquis	Kanred	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
F_2 culture—9	4	3+	0	3+	4−	3+	4	4	4−	3+	3+	1
F_3 culture—9	4	3±	0	3−	3+	3+	3+	4−	3+ to x	3	3±	1−
F_3 culture—17	4	4−	0	3+	4−	4−	4−	4	3+	3+	1	1−
F_3 culture—29	4	4−	0	3±	4−	x	x	x+	3+	3+	1±	1
F_3 culture—85	4	4−	0	3	4−	4−	4−	4−	3±	3+	x	1−
F_3 culture—149	4	x+	0	3−	x+	x	x	x	x	x	x	1−

The Effect of Inbreeding on the Pycnial and Aecial Stages

The Loss of Ability to Produce Aecia on the Barberry

The effect of inbreeding has been manifested not only in peculiarities associated with the uredial and telial stages but also in abnormalities of the pycnial and aecial stages. The loss of ability of certain strains to produce aecia and the development of uredia and telia on the barberry by some of these strains are two such abnormalities. The last-mentioned abnormality has already been reported briefly (11). As the production of uredia and telia on the barberry appears to be associated with the suppression of aecia, it is necessary to deal first with the latter phenomenon.

Strains of stem rust that have partly or entirely failed to produce aecia have been known by the writers since 1931. In that year several barberry plants were inoculated by the sporidia of teliospores of Race 1 (white), an F_2 culture derived from a cross between Races 9 (orange) and 36 (grayish-brown). The pycnial pustules resulting from these inoculations produced only traces of pycniospore-containing nectar. Although nectar was intermixed on several hundred pustules, only one of these produced normal aecia while three other pustules developed rudimentary aecia containing only a few aeciospores. Difficulties were also experienced in obtaining aecial formation by the application of nectar from other races to the pycnia of the white race. When, however, composite nectar of Race 95 was transferred to 45 pustules of Race 1 (white), aecia developed in five of these. The application of foreign nectar was therefore somewhat more efficacious in bringing about aecial formation than the intermixing of the nectar of the white race itself, but, nevertheless, the proportion of pustules producing aecia was abnormally low. Another F_2 culture derived from the same cross, Race 11 (red), behaved in a similar manner. No aecia resulted from the intermixing of the nectar of more than 50 pustules. In 1934 and 1935 a similar behavior was noted in four white races in the F_3 and F_4 generations of another cross—Race 52 (grayish-brown) × Race 9 (orange). The pycnia formed by these races produced only minute quantities of nectar which showed a tendency to dry up soon after its production. Pycniospores were present but less abundantly than in normal nectar. Such pycnial nectar as was produced by each of these races was intermixed and a record was kept of the number of pustules that produced aecia. Race 15 (white), an F_3 culture, produced aecia in only 2 out of a total of 140 pustules. Race 52 (white), another F_3 culture, produced no aecia in a total of 72 pustules. Race 9 (white), an F_4 culture, produced aecia in 13 of a total of 88 pustules. Race 57 (white), another F_4 culture, formed aecia in 17 of a total of 72 pustules.

The failure of these races to produce aecia can not, at present, be satisfactorily explained. A complete failure to form aecia, as in Race 52 (white), can not be solely attributed to the sparseness of pycniospore-containing nectar for, as many compound pustules were present, there should have been considerable aecial formation resulting from the coalescing of haploid pustules of opposite sexes even if the pycniospores were not functional. Neither can

the suppression of aecia be attributed to the loss of pigment in the urediospores, as might be concluded from the fact that most of the above-mentioned races formed white uredia. The two phenomena are not necessarily associated, as some white strains produce aecia abundantly.

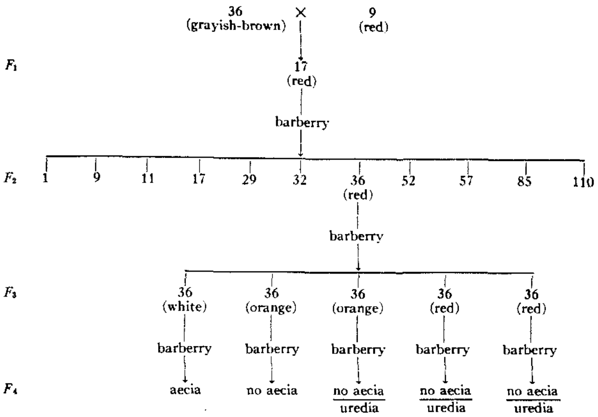
The Development of Uredia on the Barberry by Some Strains Which Have, Partially or Entirely, Lost the Ability to Produce Aecia

During the winter 1936–37, barberry plants were inoculated with five F_3 cultures derived from a cross between Races 36 (grayish-brown) and 9 (red). These five cultures were descended from the same parent, an F_2 culture which had been identified as Race 36 (red). This F_2 culture appeared homozygous for pathogenicity, as all its F_3 progeny (composed of 35 cultures) were identified as Race 36. The five F_3 cultures selected for further study differed, however, in uredial color; one being white, two orange, and two red. They differed also in their behavior on the barberry. The white culture produced pycnial nectar in abundance and formed aecia in a normal manner. The other four cultures produced very small quantities of pycnial nectar which, however, contained pycniospores, but they formed no aecia either as a result of intermixing this nectar or by the application to their pustules of the nectar of other races. One of the orange cultures, and both of the red cultures produced uredia and telia on the barberry. The interval between inoculation and the production of uredia varied somewhat in different infection trials. The shortest interval noted in any trial was 35 days, the longest 57 days, the average interval being from 44 to 47 days. Text-fig. 2 shows the origin of the cultures and the spore forms produced by them on the barberry.

In most of the infection tests, only a relatively small proportion of the pustules produced uredia, and this proportion varied considerably in different tests. In one test 50 pustules out of a total of 129 were found to contain uredia. Of these 50 pustules at least 21 bore telia as well as uredia. In a later infection trial, on a different barberry plant, uredia were found on only 10 pustules out of a total of about 150.

In certain infection tests attempts were made to determine what proportion of the uredia-bearing pustules were single and compound pustules respectively. This, however, could not be done with any degree of certainty. In the infection test mentioned above, in which 50 uredia-bearing pustules were found, it was judged that 10 of these were single pustules, 31 compound, while 9 could not be determined definitely as either single or compound. The majority of the uredia were, therefore, borne on compound pustules formed by the coalescing of two or more adjacent infections.

This raises the question of whether the uredia-bearing pustules arose from the fusion of mycelia of opposite sexes. The fact that 31 out of 60 pustules that were obviously compound produced uredia whereas 29 failed to do so would suggest that mycelia of opposite sexes interacted. On the other hand 10 pustules that were judged to be single produced uredia. It is not, however,



TEXT-FIG. 2. *Pedigree of the strains of Race 36 that produce urediospores and teliospores on the barberry.*

altogether safe to assume that these were of monosporidial origin and that the urediospores arose on homothallic mycelia.

The uredia were most abundant on the upper surface of the leaves but were not infrequently found on the lower side also. Plate I, Fig. 4 shows uredia of Race 36 (red) on the upper surface of a barberry leaf. Urediospores and teliospores were both normal in appearance. The urediospores germinated in their characteristic manner and infected wheat seedlings on which normal uredia were produced, but were unable to infect the barberry. All cultures which have, thus far, been established by these urediospores have been identified as Race 36. They are therefore pathogenically identical with the parent culture.

It is evident that in these four strains of Race 36 a reduction has taken place from a full cycle (0, I, II, III) to what might be considered a brachycycle (0, II, III). For the sake of convenience these strains will, therefore, be referred to as "brachy-strains". When viewed in this light the uredia formed by them on the barberry might be considered as primary uredia. Until these strains have been submitted to another selfing it is, however, impossible to say whether this tendency to a brachy-cycle is a permanent or a transient characteristic.

Uredia and telia have also been produced on the barberry by a culture of Race 21 collected in the field at Indian Head, Sask., in 1934. Teliospores of this culture were produced in the greenhouse in the spring of 1936 and barberries were infected by these in January, 1937. When pustules developed

on the barberry, it was observed that only about one-half of them produced pycnial nectar in a normal manner. The remaining pustules were almost white in color. Many produced no pycnia or only rudimentary ones. Some, however, contained scattered pycnia which eventually produced small quantities of pycniospore-bearing nectar. Many of the white pustules began to produce urediospores and teliospores about six weeks after inoculation (Plate I, Fig. 5). The urediospores, like the ones produced on the barberry by the cultures of Race 36, were incapable of infecting the barberry but infected wheat seedlings readily.

Three cultures were established from uredia produced on three different barberry leaves with the object of determining the physiologic races contained in them. Two of these were pure cultures of Race 1 and one was a mixture of Races 1 and 17. Races 1 and 17 also predominated in cultures originating from the aecia produced by Race 21. In a study of 36 cultures, each originating from a single aecium of Race 21, selected at random, Race 1 occurred nine times, Race 17 twenty times, Race 21 once, Race 78† five times, and race 136† twice. Although the cultures originated by the uredia were too few to permit a satisfactory comparison with those initiated by the aecia, the similarity of the physiologic races derived from the two sources would suggest that the aeciospores and the urediospores are genotypically alike.

Attempts were made to discover whether or not the pycniospores of the races that produced uredia on the barberry had lost their function. The loss of function of the pycniospores would not, it is true, explain the development of uredia on the barberry, but it might conceivably be the cause of the suppression of the aecia. If the application of nectar of these races to haploid pustules of other races resulted in the development of aecia, proof would have been obtained that the pycniospores were still functional. Accordingly composite nectar of Race 36 (red)—a race that formed uredia but no aecia on the barberry—was transferred to six haploid pustules of Race 36 (white) which has regularly produced aecia in a normal manner. When selfed, this race, like all white races, develops aecia and aeciospores of a pale buff color. When pycniospores of a red race—that is, a race producing red uredia—are used to diploidize the pustules of a white race, the resulting aecia are orange in color and contain orange aeciospores which give rise to red uredia when they infect wheat seedlings. The production of orange aeciospores in any of the six pustules to which nectar of Race 36 was applied would therefore be a proof of the functioning of the pycniospores of this race. Aeciospores of this color were, in fact, developed in four out of the six pustules. Inoculation of wheat seedlings by these aeciospores gave rise to red uredia, a fact which proved conclusively that the pycniospores of the red race, though incapable of initiating aecia in a selfing of that race, were capable of doing so when applied to the haploid pustules of the white race. Although the pycniospores were thus able to diploidize pustules of the white race, the aecia produced were

†*It should be remarked that Races 78 and 136 differ but slightly in pathogenicity from Races 17 and 1.*

scarcely more than rudimentary structures that barely succeeded in breaking through the epidermis of the barberry leaf. As the white race, when selfed, produces vigorous aecial development the lack of vigor noted in this case is clearly attributable to the red race.

In a similar manner it was demonstrated that the pycniospores produced by the white pustules of Race 21 were able to diploidize pustules of Race 1 (white). The application of a composite nectar from two pustules of Race 21 to four haploid pustules of Race 1 (white) produced orange aecia and aeciospores in all four pustules. These aeciospores, in turn, gave rise to red uredia on wheat seedlings. The four uredial cultures thus initiated were all identified as Race 17. The aecia in this instance were normal in size and structure.

The ability of the pycniospores of these strains to induce aecial formation in the white race suggests strongly that the suppression of aecia is not due to non-function of the pycniospores. This conclusion is supported by the fact that neither the pycniospores of Race 1 (white) nor those of other races appear capable of bringing about the formation of aecia when they are applied to the pycnia of the brachy-strains.

Discussion

Sufficient evidence has been presented in the present paper to demonstrate that the inbreeding of physiologic races gives rise to various abnormal characteristics that are rarely, if ever, encountered in nature. Abnormal characteristics are not, however, an inevitable consequence of inbreeding, for many inbred strains show no deviation from the normal. These abnormalities must arise in one of two ways. Either they originate through mutations that occur during the passage of the rust through the barberry, that is, during the selfing process, or they arise from a recombination of genetic factors already present in the physiologic races before they were selfed. In either case the abnormalities would be attributable to mutation. If the aberrations arise from the recombination of already existing genetic factors it would follow that these genetic factors, though present in the rust, do not produce any observable effects on the rust so long as it remains in the uredial stage. Such, indeed, would be the case if these genetic factors were recessive. They might then have originated through mutations which had taken place at some time or other in the past history of the rust; and their effects would not become visible until they had been segregated and recombined in the process of selfing.

While it is not easy to determine which of the two above-mentioned alternatives plays the more significant part in the origin of abnormal rust characteristics, it can scarcely be doubted that the process of mutation is actively at work in the rust fungi. This has been amply demonstrated in recent years by investigators of certain of the cereal rusts. Mutations for uredial color have been reported in stem rust of wheat by Newton and Johnson (9) and Waterhouse (16). Mutations for pathogenicity have been recorded by Stakman, Levine, and Cotter (15) in wheat stem rust, by Gassner and Straib (3) in *Puccinia glumarum* (Schmidt) Erikss. & Henn., and by Roberts (14) in

Puccinia triticina Erikss. An aberrant physiologic race of *P. triticina*, differing from other known races in length of incubation period, spore color, and size of uredia, has been described by Johnston (8) who suggested the possibility of its origin by mutation.

In view of the number of authentic mutations already recorded in cereal rusts, it seems probable that mutation in the rust fungi is a constantly recurring phenomenon which plays a part in the origin of new genetic factors that do not always find expression while the rust remains in its dikaryotic (uredial) stage. As, however, stem rust is a heterothallic rust, a continuous interchange of genetic factors is taking place between the various races of which it is composed, an interchange accomplished by the intermixing of the pycnial nectar of different haploid pustules and the fusion of pustules arising from mycelia of opposite sexes (1). Consequently most physiologic races are in a heterozygous state. This heterozygosity has probably been increased by numerous mutations of a recessive type, which have taken place in the past, and which have been distributed among physiologic races through the processes just indicated. It is probable that many of the abnormalities that have appeared through the selfing of physiologic races are the result of such recessive mutations which are masked by dominant factors, and thus do not produce visible effects until they are brought together in a homozygous condition. It has been demonstrated in crossing and selfing studies that some of the abnormalities reported in the present paper behave as if they were governed by recessive factors. Thus grayish-brown and orange urediospore color is dominant over white, whereas red spore color is dominant over grayish-brown, orange, and white. While the inheritance of other abnormalities, such as the inability of the uredia of certain strains to rupture the wheat epidermis, has not been studied in detail it would seem probable, owing to their not infrequent occurrence in the selfing of perfectly normal rust strains, that they are also governed by recessive genetic factors. If this assumption is correct and generally applicable to abnormalities in stem rust, it would follow that the abnormal strains are homozygous for the genetic factors responsible for their deviation from the normal. In the laboratory this homozygosity is accomplished by inbreeding for several successive generations, a process which probably occurs rarely in nature. When abnormal types of rust do occur in nature they probably have a low survival value and would not be observed frequently.

The production of urediospores and teliospores on the barberry is, perhaps, the most unexpected of the abnormalities described in this paper. Any attempt at explaining this phenomenon must take into account the suppression of aecial formation which, thus far, has always accompanied it. One possible reason for the failure of a race to produce aecia would be the non-function of the pycniospores. It has been pointed out that the brachy-strains of Race 36 cannot be induced to form aecia through either an inter-mixing of their own nectar or the application of the nectar of other races. Nevertheless, the nectar from these strains, when applied to haploid pustules of other races, is capable of bringing about diploidization which results in some aecial formation.

The same holds true for the pycniospores of the very sparse nectar of the white pustules of Race 21. Obviously, therefore, the pycniospores are still functional.

Another possible reason for the failure of these strains to form aecia might be the loss of a sex factor. However, if the factor for one sex were lost the application of a composite nectar of other races—nectar containing pycniospores of both sexes—should bring about aecial formation in approximately one-half of the haploid pustules, which, as already pointed out, does not occur. The factor for at least one sex must be present, otherwise the pycniospores of the brachy-strains would not be capable of diploidizing the haploid mycelia of other races.

It would seem then that the only explanation that can be advanced is to assume that a genetic factor (or factors) governs the production of aecia and to ascribe the failure of aecial formation in these strains to a loss of this factor or factors. That this factor acts as a dominant is suggested by the fact that the pycniospores of the brachy-strains do not suppress aecial formation when they are used to diploidize haploid pustules of another race. The lack of ability to produce aecia is, therefore, a recessive condition, or at least not a dominant one. With the loss of the factor governing aecial production, sporulation takes the alternative form and uredia are produced.

Regarded from the point of view of phylogeny the occurrence of uredia and telia of *Puccinia graminis* on the barberry may have some significance in explaining the origin of short-cycled rusts from heteroecious long-cycled forms. According to Jackson (5) microcyclic forms arise in most cases from the haploid generation of heteroecious eu-forms through the replacement of the aecia by telia. Short-cycled species which appear to have originated in this manner are known as "correlated" species. Thus, for example, *Puccinia Grossulariae* (Pers.) Lag., with aecia on *Ribes* and uredia and telia on *Carex*, may have given rise to *Puccinia Parkerae* Diet. and Holw., a micro-form with telia on *Ribes*. Similarly, as pointed out in private correspondence by Dr. G. R. Bisby, *Puccinia graminis* might, in theory, have a microcyclic, correlated species with telia on *Berberis vulgaris*. The development of uredia and telia on the barberry by Races 36 and 21 may perhaps be considered as a tendency on the part of *Puccinia graminis* towards the formation of such a correlated species. This interpretation would lend support to the theory that correlated microcyclic forms were derived, through reduction, from heteroecious long-cycle forms.

It appears doubtful whether the occurrence of uredia and telia on the barberry throws any light on the relative primitiveness of heteroecism and autoecism. A heteroecious rust has apparently made an attempt—but an unsuccessful one—to become autoecious; unsuccessful because the urediospores produced on the barberry still retain their normal physiological nature and infect grasses, but not the barberry. It might be argued that this phenomenon supports the idea that heteroecism is a more primitive condition than autoecism, that this attempt at autoecism represents an inherent tendency in

heteroecious rusts to become autoecious. But possibly it might also be argued that a heteroecious rust was here attempting to revert to a more primitive autoecious condition.

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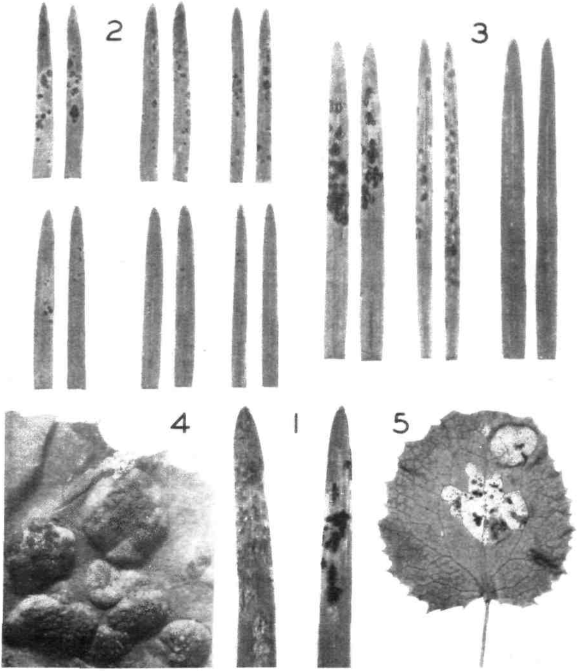

FIG. 1. *Infection types produced on wheat seedlings by two physiologic races of P. graminis Tritici. Left: "Subepidermal" pustules produced by Race 17 (cinnamon brown). Right: Normal pustules produced by Race 56 (red).* FIG. 2. *Infection types produced on seedlings of three wheat varieties by two cultures of Race 149. Top row: left to right, Marquis, Spelmar, and Einkorn infected by Culture No. 9. Bottom row: left to right, the same varieties infected by Culture No. 41 which produces a less vigorous pustule development.* FIG. 3. *The effect of high temperatures (mean maximum 99.6° F., mean minimum 66.8° F.) on the infection types produced by two physiologic races of P. graminis Tritici on Little Club seedlings. Left: Race 48—unaffected by the high temperature. Right: Race 36 (Sudan Brown)—strongly affected by the high temperature. Centre: Race 36 (Sudan Brown)—at approximately normal temperatures.* FIG. 4. *Upper surface of a barberry leaf showing uredia on pustules of P. graminis Tritici, Race 36 (red). Enlarged.* FIG. 5. *Lower surface of a barberry leaf showing a compound pustule of P. graminis Tritici Race 21. One component of the pustule contains aecia, the other small uredia.*

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STUDIES ON *RHIZOCTONIA SOLANI* KÜHN.

III. RACIAL DIFFERENCES IN PATHOGENICITY[1]

BY G. B. SANFORD[2]

Abstract

Pathogenicity tests on potato stems were made of 133 isolates of *Rhizoctonia Solani* Kühn. Of these, 114 were from random sclerotia on random tubers from four fields, 13 from lesions on potato stems, and 8 from single basidiospores. A number of tests were made in the laboratory at 17° and 23° C., in two contrasting types of artificially infested, unsterilized, virgin soil, which was maintained at optimum moisture content for disease expression.

More of the isolates were pathogenic in the infertile podsol soil than in the fertile black loam. Eighteen per cent of the isolates were of virulent rank in the latter soil, in contrast to 34% of them in the former one.

Indications from the study were that, under average soil conditions, approximately 20 to 50% of the isolates of *R. Solani* from sclerotia on random tubers may be assigned to the zero and marginal classes of pathogenic rank. The data also indicated that certain isolates were inherently very deficient in pathogenicity to potato stems, while others characteristically possess a high degree of virulence. Thus, with regard to the effect of soil type and racial differences in pathogenicity, it would appear that the results of this study help to explain why the stems of a high percentage of plants from sclerotia-infested sets often escape with little or no infection under field conditions.

In a previous study (14), involving 34 tests of an experiment made in different fields, an average of about 42% of the 3,400 plants, which grew from sets heavily infested with sclerotia of *Rhizoctonia Solani*, had lesions on the stems at the end of six weeks. But when a deduction was made for the infection from the soil, an average of only approximately 29% of the plants had lesions of any size, attributable to the sclerotia on the sets. In only four cases did the sclerotia on the sets augment the percentage of infected plants by more than 50%. In approximately one-third of the tests the indicated increase fell below 20%. Finally, in those soils apparently having a relatively light natural infestation, the increases produced by the sclerotia-infested sets were extremely variable and certainly less severe than what might be expected. The following paired results, which are taken from Table I (14), will illustrate this: 98-81; 95-25; 99-32; 96-24; 91-31; 86-50; 80-15; 90-21; 95-37 and 81-42. In each case the first figure given represents the percentage of plants with clean stems from clean treated sets, and the second one the percentage of infected plants for which the sclerotia-infested sets were responsible.

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Contribution No. 529 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, co-operating with the Department of Field Crops, University of Alberta.

[2] *Pathologist-in-Charge, Dominion Laboratory of Plant Pathology, Edmonton, Alberta.*

The unexpectedly low effectiveness of the sclerotia on the sets was not explained by the data available. However, it seemed that one of several contributing factors might be the presence of a large proportion of sclerotia of marginal pathogenicity.

This paper will be confined to a study of the relative disease-producing ability on potato stems of 133 isolates of *R. Solani*, as obtained from the potato plant in six different fields in the Edmonton district.

Literature Review

Braun (1), in 1930, reviewed the literature relating to racial specialization in *R. Solani*, as indicated by morphological, cultural and host reaction phenomena. Therefore, only a few typical references are necessary here.

Matsumoto (6) secured, with Isolate P, infection of *Solanum tuberosum*, *S. melongena*, *Lactuca sativa*, *Phaseolus vulgaris* and *P. lunatus*. He states: "The pathogenic capacity of the strains is always manifest when inoculation is made on plants belonging to the same species of host as that from which the culture originated".

LeClerg (4), 1934, concluded that rhizoctonia root rot of sugar beets in field culture is probably caused by strains distinct pathogenically from those which attack the potato plant; also that groups of isolates from the lesions on stems of potato, and those from the diseased tissue of sugar beets were about equally destructive in damping off alfalfa seedlings.

Peltier (9), 1916, concluded that the many isolates which he obtained from a wide range of hosts were able to attack the same species of plant, and that no marked host specialization was noted in any of his isolates.

With regard to racial characteristics other than pathogenicity, Matsumoto (6), 1921, found that certain isolates differed from others in enzyme activity and in ability to utilize potassium nitrate. Edwards and Newton (2), 1937, reported that isolates from the same or from different hosts might express either different or similar enzyme activity. They did not report the relation of enzyme activity of several isolates to their relative virulence on a given host.

Racial differences in growth, as affected by temperature, have been reported by Matsumoto (6), Monteith and Dahl (7), LeClerg (4), Rosenbaum and Shapovalov (12) and others. For growth, the most common optimum appears to have been between 22° and 25°, while for a relatively smaller number of isolates it was somewhat higher than 25° C., or slightly lower than 22° C. Richards (11) obtained growth from 4.6° to 32.5° C. However, for greatest pathogenicity to potato, bean and pea, the optimum was approximately 18° C., while for the isolates pathogenic to the sugar beet root, LeClerg (4) concluded that a soil temperature of approximately 25° to 33° C. was best. For carnation rot Peltier (9) found temperatures above 88° F. most favorable.

Certain other factors are said to affect the virulence of the pathogen. Rabinovitz-Sereni (10), Gratz (3), Weber (15), Samuel and Garrett (13), and

others have found the optimum hydrogen ion concentration for growth to lie between pH 6.5 and 7. The effect of age and condition of culture have been discussed by several workers, but without general agreement as to whether a young culture is more virulent than an old one. Wellensiek (16) found the virulence of a pure culture unchanged after eight years. Peltier (9) concluded that a soil moisture content either too low or too high for best growth of the carnation plant favored the disease, if the temperature was above 88° F. Müller (8) reported that rhizoctonia injury to potato was increased in a wet soil, while Martin (5) observed that it decreased as the soil moisture content increased.

With regard to soil type Müller (8) found that the vegetative growth of *R. Solani* was abundant and rapid in a rich loam, but restricted and slow in a sandy, infertile soil. He argued that the former type of soil, by providing a more abundant and rapid growth of the pathogen, would be more favorable to development of the disease than the latter type.

Experimental Methods

Numerous preliminary trials were made to obtain a satisfactory method to test the disease-producing ability of the isolates. Attention was given to type of soil, soil temperature, soil moisture, method of infesting the soil, and the time required for an adequate test.

Soil Type

Two contrasting types of virgin soil were chosen, and each used with its natural microflora, except for the addition of a small fraction of soil inoculum. One was the black organic loam (Type I) common to the park area of Alberta, and the other a white podsol (Type II), characteristic of the wooded areas of certain parts of Alberta. Wyatt and Newton (17) have provided a description of the physical properties, and also a chemical analysis of the A, or cultivated, horizon of each of the two types mentioned. Following are the data: Type I, nitrogen 0.53, phosphorus 0.10, calcium 0.94, magnesium, 0.50, soil reaction pH 6.4; Type II, nitrogen 0.09; phosphorus, 0.06, calcium 0.55, magnesium 0.33, soil reaction pH 6.2.

Soil Moisture and Temperature

At the beginning of the test the artificially infested soil was made up to approximately 29% of its moisture-holding capacity for Type I, and 27% for Type II. This moisture content was what might be considered optimum for each soil. Water was not added during the test. Under the conditions of the experiment the moisture lost during the test varied between 1 and 1.5%.

Two soil temperatures were used for testing each isolate, namely 16°–17° C., and 23°–25° C., but usually 17° and 23° C.

The Experiment

Each isolate was increased in steam sterilized soil without additional nutrient. This pure soil inoculum was mixed in the proportion of one part of inoculum to 15 parts of natural soil, made up to the required moisture content.

A potato set was placed on a small amount of this soil at the bottom of each one-litre Erlenmeyer flask, and covered to a depth of approximately six inches. Cotton plugs were inserted in the flasks. The flasks were then placed at the required temperature for 21 days.

The variety used was Early Ohio, which is known to be very susceptible to rhizoctonia stem canker. The tubers from which each of the sets was cut were from one lot kept in cold storage until required. Fifteen sets were used for each test.

At the end of 21 days, when the sprouts had emerged from the soil, a numerical disease rating was given for each unit, and the length of the sprout measured. Also, the massing of the hyphae and the formation of sclerotia on the stem or set, if any, were recorded. The soil moisture was determined at this time. The infections, expressed in percentage, for each isolate for each type of soil, and for the two temperature series, are listed in Table I.

The Isolates

The six groups of isolates tested were secured from six different sources. In four of the six groups (A, B, C and D) each isolate was obtained from one sclerotium on one tuber, and each tuber was taken from a separate and random hill in the potato field. In another case, they were taken from rhizoctonia lesions on different potato stems. Definitely monosporus cultures from basidiospores of the *Corticium* stage produced on potato stems comprised Group F. In isolating these monosporous cultures it was absolutely necessary to decide by careful microscopic examination whether the growth on nutrient agar below the suspended hymenial structure was from definite basidiospores or merely from hyphal fragments which were frequently set free.

With the exception of the isolates from the basidiospores, any of the other isolates might be, in a sense, mass cultures, even after repeated transfer in pure culture. Moreover, isolates from sclerotia on tubers, as well as many of them from stem lesions, are merely representative of the fungus as distributed in the soil of a given field at the time the sclerotia formed, and not necessarily pathogenic forms. The isolates, as indicated, were considered more suitable for the immediate purpose of the study than hyphal-tip isolates would be.

Results

The experimental results, which are listed in Table I, indicate the relative disease-producing ability on potato stems, during 21 days, of each of the 133 isolates tested. Detailed records prove that throughout these tests there was always adequate growth, on the potato stems, of hyphae of all isolates to produce disease. It is important to observe in Table I the percentage of stems which escaped attack, since this seems to be closely related to the pathogenic ability of an isolate. For the convenience of the reader, the data in Table I have been summarized in Table II to indicate the percentage of isolates pathogenic, and the relative severity of disease in each of the two contrasting soil types. In Table III the data are again arranged to show the percentage of isolates of each series according to degree of virulence. The illustrations

in Plate I indicate the inherent ability of different isolates to produce severe, medium, light, or no disease.

Effect of Soil Type

A comparison of the data in Table I, and also in Table II, leaves no doubt that in the first test, at least, a distinctly higher proportion of isolates were pathogenic in Soil II than in Soil I, and also that, in general, the isolates

TABLE I

PATHOGENICITY OF 133 ISOLATES OF *Rhizoctonia Solani* FROM SIX DIFFERENT SOURCES, AS INDICATED ON POTATO STEMS IN TWO CONSTRASTING TYPES OF SOIL AT APPROXIMATELY 17° C.

No.	Soil I				Soil II	
	17°		23°		17°	
	Rt.†	*	Rt.†	*	Rt.†	*
(Series A)‡						
1	41	25	66	0	51	6
2	43	12	32	31	70	0
3	33	25	7	81	36	19
4	11	62	12	62	0	100
5	0	100	0	100	13	44
6	28	6	18	31	32	31
7	7	75	19	25	16	75
8	27	44	31	38	54	6
9	0	100	0	100	8	81
10	0	100	27	31	0	100
11	18	31	7	81	16	62
12	0	100	2	88	2	75
13	16	62	44	6	53	6
14	8	81	28	31	60	0
15	26	50	32	31	60	0
16	0	100	0	100	2	94
17	0	100	0	100	26	19
18	0	100	0	100	35	19
19	1	88	0	100	27	44
20	45	25	4	19	20	44
21	1	81	0	100	39	12
22	0	100	0	100	50	6
23	0	100	0	100	28	44
24	0	100	0	100	28	19
25	0	100	0	100	31	0
(Series B)‡						
1	36	31	10	81	60	0
2	32	31	24	50	62	0
3	2	81	0	100	46	6
4	44	19	26	50	61	6
5	0	100	0	100	5	81
6	0	100	0	100	11	81
7	10	62	10	56	67	0
8	0	100	0	100	10	57
9	5	81	4	88	27	56
10	30	44	10	75	45	31
11	0	100	0	100	12	94
12	0	100	0	100	11	69
13	0	100	0	100	31	31
(Series B—*Concluded*)						
14	0	100	0	100	25	38
15	0	100	0	100	37	31
16	0	100	0	100	15	75
17	6	81	6	94	37	38
18	0	100	0	100	10	81
19	0	100	0	100	11	75
20	0	100	0	100	5	88
21	0	100	0	100	3	94
22	0	100	0	100	49	6
23	0	100	0	100	47	12
24	0	100	0	100	22	44
25	0	100	0	100	30	50
(Series C)‡						
1	3	75			16	69
2	0	100			80	0
3	0	100			41	31
4	4	75			21	56
5	0	100			16	62
6	0	100			0	100
7	0	100			2	88
8	0	100			12	62
9	0	100			1	88
10	0	100			4	69
11	0	100			0	100
12	0	100			36	19
13	0	100			70	0
14	10	81			11	69
15	7	81			0	100
16	0	100			0	100
17	0	100			12	75
18	0	100			25	50
19	1	94			13	69
20	0	100			36	31
21	0	100			1	56
22	0	100			13	50
23	2	88			14	75
24	0	100			12	44
25	0	100			8	44

† *Disease rating %.* * *Per cent plants not lesioned.*
‡ *A, B and C, from random sclerotia on potatoes in field.*

TABLE I—*Concluded*

PATHOGENICITY OF 133 ISOLATES OF *Rhizoctonia Solani* FROM SIX DIFFERENT SOURCES, AS INDICATED ON POTATO STEMS IN TWO CONTRASTING TYPES OF SOIL AT APPROXIMATELY 17° C.—*Concluded*

No.	Soil I				Soil II			
	Test 1		Test 2		Test 1		Test 2§	
	Rt.†	*	Rt.†	*	Rt.†	*	Rt.†	*
(Series D)‡								
1	0	100	18	70	54	10	30	50
2	0	100	0	100	0	100	0	100
3	52	0	70	0	70	0	63	10
4	0	100	0	100	0	100	0	100
6	0	100	20	10	3	90	0	100
7	0	100	46	50	0	100	0	100
8	44	0	49	10	62	0	0	100
10	0	100	6	80	0	100	0	100
11	0	100	0	100	0	100	0	100
12	2	90	0	100	51	20	60	40
14	0	100	0	100	10	70	16	70
15	0	100	0	100	0	100	1	100
16	0	100	2	80	0	100	0	100
17	5	90	10	60	0	100	0	100
18	0	100	0	100	7	80	5	90
19	0	100	1	80	0	100	14	80
20	54	0	45	20	60	0	16	0
21	6	0	7	70	9	90	20	70
22	0	100	0	100	0	100	1	100
24	7	80	60	0	40	30	60	10
25	9	60	60	20	30	40	60	0
26	20	0	0	100	10	80	0	100
28	15	20	18	40	60	0	27	60
29	0	100	0	100	0	100	0	100
30	0	100	13	70	0	100	2	90
31	52	0	55	0	51	10	45	20
35	30	0	30	40	5	80	30	30
36	0	100	0	100	0	100	0	100
38	30	0	50	0	40	20	40	10
39	0	90	32	40	3	60	6	90
42	0	100	31	40	5	80	20	60
43	0	100	50	0	0	100	0	100
44	0	100	4	90	0	100	0	100
45	0	100	3	90	0	100	0	100

No.	Soil I				Soil II			
	Test 1		Test 2§		Test 1		Test 2§	
	Rt.†	*	Rt.†	*	Rt.†	*	Rt.†	*
(Series D—*Concluded*)‡								
47	0	100	0	100	0	100	1	70
48	0	100	0	100	0	100	0	100
49	8	0	0	100	0	100	0	100
(Series E)‡								
70	0	100	0	100	40	30	0	0
71	0	100	0	100	0	100	0	0
72	18	0	8	80	0	100	0	0
73	0	100	0	100	3	40	8	90
74	0	100	0	100	0	100	0	0
75	0	100	0	100	0	100	0	0
76	40	30	50	10	60	0	55	10
77	0	100	34	40	45	20	17	60
81	0	100	0	100	0	100	0	0
82	0	100	0	100	6	90	25	40
83	0	100	0	100	0	100	0	0
85	0	100	5	80	0	100	0	0
86	0	100	0	100	0	100	0	0
(Series F)‡								
105	0	100	0	100	0	100	0	100
106	13	80	11	80	46	20	10	70
107	0	100	0	100	30	50	0	100
108	0	100	56	0	63	0	0	100
109	0	100	0	100	12	70	0	100
110	1	90	34	40	40	30	0	100
111	0	100	0	100	37	30	0	100
112	2	90	0	100	30	50	0	100

† *Disease rating %.*

‡ *A, B and C, from random sclerotia on potatoes in field. D, from random sclerotia on potatoes in field; E, from random lesions on potato stems in field; F, isolates from single basidiospores.*

§ *Second test made by replanting soil of first test.*

* *Per cent plants not lesioned.*

produced more disease in the former soil. The percentage of isolates pathogenic in the four tests of all series in Soil I was 34, while for Soil II it was 73. For severity of disease the corresponding figures are 6% and 22%, respectively. The detailed data given in Table I confirm the trend just indicated. This general tendency has also been observed in a number of other experiments not reported here. Owing to the operation of certain factors, which apparently

are not significantly affected by either soil temperature or soil moisture content, the disease expression in either soil may be slight or great. Whatever the cause may be, all isolates appear to respond in a given test in the same general direction of disease expression. Also, as a rule, the trend is indicated both by the percentage of isolates pathogenic and by the numerical disease rating. For example (Tables I and II), when the two soils were replanted immediately after the first test had been completed, the difference just indicated occurred in the second test in both soils. In this second test a number of isolates which expressed slight or no pathogenicity in Soil I in the first test produced more disease, and in Soil II certain erstwhile marginally pathogenic isolates failed to produce disease. The percentages of the isolates pathogenic in the first tests in Series D, E and F for Soil I were 38, 8 and 38, and for the second test 62, 25 and 38; for the first test in Soil II, 49, 33 and 87, and for the second test 51, 25 and 13.

Percentage of Isolates which Produced Disease

The ability of an isolate to produce disease was judged by its performance in one or more pathogenicity tests which were made in two contrasting types of soil, and some of them at two temperatures, *viz.*, 17° and 23° C. For example, isolates Nos. 1–50, inclusive, received three tests; Nos. 51–75, inclusive, two tests; and the remaining 57 isolates four tests at 17° C. The data in Table I emphasize that it would be very unsafe to rely on one test, no matter how well it was replicated or how favorably the soil temperature and moisture were maintained. Apparently there are certain isolates which are fairly constant in producing a substantial, although often variable, amount of disease, while others tend to be marginal, regardless of soil type, and, finally, certain ones appear to lack the ability to cause disease, even under a fairly wide range of

TABLE II

AN ANALYSIS OF THE PATHOGENICITY OF 133 ISOLATES OF *Rhizoctonia Solani* ON POTATO IN TWO CONTRASTING SOIL TYPES, ACCORDING TO SOURCE OF ISOLATE AND AVERAGE DISEASE RATING

Series	%* isolates producing disease						Av. disease rating % in Test 1		
	17° C.				23° C.	†			
	Soil I		Soil II		Soil I		Soil I		Soil II
	Test 1	Test 2	Test 1	Test 2	Test 1		17° C.	23° C.	17° C.
A	56		92		56	100	12	14	30
B	32		100		28	100	7	4	30
C	24		84			88	1		17
D	38	62	49	51		84	9		15
E	8	25	33	25		54	4		12
F	38	38	87	13		88	2		32
	34‡	50§	73‡	40§		86‡	6‡		22‡

* *To nearest decimal.*
† *Pathogenic in at least one of several tests.*
‡ *Percentage of 133 isolates.*
§ *Percentage of 58 isolates, as given in both soils of Test 1, replanted.*

conditions. All isolates, whether usually very pathogenic or those of marginal nature, evidenced more or less erratic behavior, and although it is not entirely evident from these data, there is indication that the isolates of marginal pathogenicity are the most erratic.

TABLE III

AN ANALYSIS OF THE PATHOGENICITY OF 133 ISOLATES OF *Rhizoctonia Solani* AS EXPRESSED ON POTATO STEMS. THE PERCENTAGE OF ISOLATES IN EACH VIRULENCE CLASS IS GIVEN FOR EACH OF THE SIX SOURCES IN TWO SOIL TYPES

Series	Soil I				Soil II			
	Zero	Marginal	Intermediate	Virulent	Zero	Marginal	Intermediate	Virulent
	0	1–15	16–30	31+	0	1–15	16–30	31+
A	36	16	24	24	8	16	28	48
B	68	16	4	12	0	36	20	44
C	76	24	0	0	16	48	16	20
D	30	27	13	30	38	24	11	27
E	69	8	8	15	61	8	8	23
F	50	25	0	25	13	0	37	50
*	52	20	10	18	22	26	18	34

* % of 133 isolates.

The data in Table II illustrate in summary form the variability in pathogenicity which occurred between the first and second tests of the isolates, and also the effect which a different soil type may exert on it. If a disease rating of form 1–15%, inclusive, represents disease-producing ability of marginal rank, 16–30%, intermediate rank, and 31+%, virulent rank, the 133 isolates of the five series can be classified on the basis of performance in all tests given them in both soil types (Table III). Thus, for Soil I, 52% of the 133 isolates produced no disease, while 20%, 10% and 18% of them were of marginal, intermediate and virulent rank, respectively; while for Soil II, only 22% of them failed to produce disease, and the corresponding percentages were 26, 18 and 34, respectively. The percentage of isolates of virulent rank in Soil I was 18, and in Soil II, 34. Fourteen per cent of the isolates produced no disease in any test made in either soil. The practical bearing to the rhizoctonia disease problem of a relatively high percentage of isolates having little or no ability to produce disease, and a relatively low percentage of them having virulent rank, will be referred to later.

Source of Isolate

One purpose of this study was to learn whether isolates, as derived at random from one field or source, may be more virulent than those from another. A comparison of the relative disease-producing ability of the isolates from the five series is available in Tables II and III. The percentage of isolates in each series, which produced disease in one or more tests in both soils, is listed in Column 7 of Table II. The 25 isolates each of series A and B

all produced disease, while 88% of the 25 in Series C, 84% of the 37 in D, 54% of the 13 in E, and 88% of the 8 isolates in Series F produced more or less disease. Perhaps only in Series A, B, C and D was the number of isolates adequate, because it is evident from these studies that definite conclusions with regard to pathogenic ability of a group of isolates cannot be made without testing a fairly large number, and repeating the test under different conditions. Yet, one cannot ignore the apparently significant trends in the data which suggest that not only may there be a different percentage of the isolates from one source pathogenic than from another, but that there may also be a difference in the proportion of isolates of virulent rank to those of intermediate or marginal rank in each case. These trends are also illustrated by the data in Table III, where, under a slightly different arrangement of the isolates of each series according to their disease-producing ability, rather marked differences appear. For example, in Series C, Soil I, none of the isolates ranked as virulent or even as intermediate, and a similar tendency also occurred again in Soil II. Also, in Series A and F, of Soils I and II, there were correspondingly more isolates of virulent rank than in the other series. Series F (Soil II), having 50% of the isolates of virulent rank and 37% of intermediate rank, is of special interest, because these isolates were from single basidiospores of the *Corticium* stage. Of the 13 isolates of Series E (from lesions on potato stems) 69% failed to produce disease in Soil I, and 61% in Soil II. The corresponding percentages for Series A, B, C, D and F in Soil I are 36, 68, 76, 30 and 50, respectively; and in Soil II, 8, 0, 16, 38 and 13, respectively. The obvious conclusion from these data is that without a thorough test one cannot assume that an isolate from a lesion is pathogenic merely because it had its origin there. But, apart from this consideration, there does appear the possibility that, in general, the sclerotia on tubers from one field may be less able to produce disease than those from another field. The situation with regard to the relative disease-producing ability of isolates obtained from basidiospores as compared to that of those from sclerotia cannot be decided from the data given. However, it was proved that spores from the *Corticium* stage may yield a very high percentage of virulent isolates, and also, like cultures from sclerotia, may yield isolates which ordinarily fail to produce disease. A study of the relation of the *Corticium* stage to the disease problem appears to merit more careful study than has yet been given to it.

Discussion

The main points of interest emphasized by the data presented are that in nature *R. Solani*, as obtained from the potato plant, commonly consists of many forms or races, which differ widely in ability to produce lesions on potato stems, and that, among other possible factors, this ability may be influenced very definitely by the type of soil in which the test is made. Obviously, these two characteristics have an important relation to the problem of disease control.

No satisfactory explanation is available from the data for the marked differences in disease expression of the isolates in the two soil types. According to Wyatt and Newton (17) the nitrogen in Soil I was approximately six times greater than in Soil II, while the phosphorus, calcium and magnesium were each nearly twice as much. Whether the marked difference in disease response was associated with these differences, or with the dissimilar physical nature and microflora of the two soils is unknown. The data indicate that the sets furnished ample food for the host in both soils during the short period of test. Thus, it seems probable that the difference in disease expressed can be explained by a change in the pathogen in each case, and not by a change in the host. The growth of the isolates was sufficiently abundant in both soils to cause a maximum of disease, if other conditions were favorable. Therefore, contrary to Müller (8), it seems questionable whether the actual relation between either a rapid or an abundant growth of the pathogen and its ability to produce disease is sufficiently well understood to postulate the effect of these factors under natural field or laboratory conditions. Despite the strong and constant tendency in the first planting for less disease in Soil I than in Soil II (Table I), the expression was at times similar in the two soils, or even less in Soil II than in Soil I, although the temperature and moisture factors were the same. Thus, it would appear that certain obscure factors may operate to counteract the usual effect of a given soil type.

The data in Table I show that, ordinarily, a collection of isolates made at random either from sclerotia on tubers, lesions on stems, or from basidiospores in any field, may consist of a surprisingly high percentage which are nonpathogenic or weakly pathogenic, and apparently a relatively low percentage of those which may, under ordinary conditions, be classified as really virulent. Of the 133 isolates tested in this study, those with no pathogenicity, and those of marginal rank in Soil I made up 72% of the total, and 48% when tested in Soil II (Table III). In Soil I, 18% of them exhibited virulent rank, and in Soil II, 34% of them. Thus, it seems that this information helps to explain why, in the 34 field experiments previously reported (14), the stems of an average of approximately 29% of the 3,400 plants from sclerotia-infested sets had no lesions. The fact that there was more than one sclerotium on each set planted might introduce a difficulty in this explanation, especially if the majority of the sclerotia on each set were of different origin. However, if the majority of them were of one origin (or consisted of weakly pathogenic strains) there would be, as these studies indicate, many cases of escape. The additional possible effect of soil type in a field has already been referred to.

The sclerotia on the tubers should be a fairly representative sample, in disease-producing ability, of *R. Solani* as it exists in the soil of the field when the sclerotia are formed. Obviously, sclerotia may be abundant or extremely scarce on the tubers, irrespective of the presence or absence of disease lesions on the plant. If much disease is present in a given field, it is logical that not only have the soil and other conditions been favorable, but that virulent races are relatively abundant. Hence, sclerotia from such a field might tend,

PLATE I

PLATE I. *Each group represents the inherent ability of different isolates of Rhizoctonia Solani to canker potato stems.*

if introduced on untreated tubers to another field, the population of which consisted of relatively few virulent races, to increase permanently in the new field the proportion of virulent to non-virulent forms. The results of this study suggest that the sclerotia from one field may be more dangerous than those from another. For example, the isolates of non-pathogenic and marginal rank from Field C comprise 100% of that group in Soil I, and 64% in Soil II. Of those from Group E (lesions on stems) the corresponding percentages for Soils I and II were 77 and 69. For Groups A and D, the percentages were 52 and 57, respectively, in Soil I, and 24 and 62, respectively, in Soil II. The percentage of the isolates in Groups A and F which were of virulent rank in Soil I, were 24 and 25, respectively, and in Soil II, 48 and 50, respectively.

The data, based on only 10 samples, indicated that a fairly large proportion of the virulent isolates might arise from the *Corticium* stage. In the field, the *Corticium* stage may or may not be associated with diseased plants, or it may be very prevalent in a field, the potato plants of which are particularly free from disease lesions. Consequently, it is an interesting and important question whether the *Corticium* stage tends to augment even temporarily the percentage of virulent forms.

Further, one asks whether some or all of those isolates apparently not pathogenic to potato in these tests could be induced to attack it by reasonable manipulation of the host, the fungus, or the environment. According to Matsumoto (6) all isolates from potato should attack this host, and Peltier (9) concluded that even though the individual isolates from many different species differed in virulence, all were pathogenic to carnation, if the conditions were made favorable enough for the disease. In the present study 14% of the isolates from potato plants appeared unable to attack the potato under very favorable conditions.

In conclusion, the results of this study are of value in that they present a general idea of the relative ability of random isolates of *R. Solani* from the potato plant to attack the host under normal conditions. However, the data do not indicate what proportion of the 133 isolates might attack (excluding the damping off phase) other economic field crops which may follow a severely infested crop of potatoes. If, as LeClerg (4) suggests, certain isolates are rather specific to the sugar beet root, a further analysis of the general problem might reveal much needed information.

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STUDIES OF THE TOMATO IN RELATION TO ITS STORAGE

I. A SURVEY OF THE EFFECT OF MATURITY AND SEASON UPON THE RESPIRATION OF GREENHOUSE FRUITS AT 12.5° C.[1]

BY E. J. M. WALFORD[2]

Abstract

Tomatoes were grown in the greenhouse at different seasons of the year, individual fruits were picked at various stages of maturity and continuous records of their respiration obtained at 12.5° C. It was found that the fruits of the late spring and summer went through the customary series of extensive changes in respiration rate as they ripened at the low temperature, and exhibited the lack of durability normal to this fruit. In contrast to this, the fruits of the late autumn, winter and early spring, if picked before the external appearance of red pigment, passed into a stable state in which ripening proceeded with but little change in respiration rate and with greatly enhanced duration of life at 12.5° C.

The tomato fruit has been investigated with a view to storage much less than the dessert fruits, doubtless because so large a portion of this crop is processed by canners. Nevertheless important quantities, and especially the greenhouse crops, are consumed fresh, and the storage properties of this fruit are of importance in its marketing and transportation. In the future we may anticipate that, as with other fruits, the problems of storage will come to influence even culture and harvesting practice.

Experience shows that the tomato's natural lack of durability is not easily overcome by low temperature. The fruit tends to be intolerant of the severe retardation of its ripening processes by temperatures near 0° C., and to break down rapidly upon removal to higher temperatures after quite short exposures to low temperature (9). The consequence is that moderate, compromise temperatures in the vicinity of 10° to 12° C. are most commonly advocated for field-grown tomatoes in temperate regions (1, 9), though somewhat lower temperatures appear to be permissible for tomatoes grown in the tropics (8). Moderate temperatures usually mean short-term storage for the purpose of increasing the flexibility of marketing, rather than for preservation beyond the season.

The outlook for long-term storage is not very encouraging. Yet it is known from farm experience that late tomatoes removed to a suitably cool place in the autumn may be held, ripening gradually and yielding a supply of marketable fruit, well on into the winter. This suggests that the seasonal factors operative during the growth of the fruit may be effective in influencing durability. There is reason to believe also that growth conditions may be so adjusted as to diminish the intolerance of the tomato to low temperature.

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[2] *Formerly Research Assistant, Department of Horticulture, O.A.C., Guelph; present address, Terminal Warehouses Ltd., Toronto.*

For instance, Barker (1) found that temperatures below 15.5° C. were injurious to English hothouse tomatoes, whilst Wardlaw and McGuire (8) showed that their tropical fruit was better preserved at about 5° C. Kidd and West (6) later discovered that English summer-grown fruit tends to behave in respect of temperature in the manner of the tropical tomatoes, whereas English autumn-grown fruit gave results such as those obtained by Barker. Evidently there is something to be learned about the influence of season upon the properties of the fruit that concern its preservation in the living state.

Consequently, when it was decided in 1934 to begin the study of tomatoes in relation to their storage, it was felt that one of the most useful additions to our knowledge would be attained by systematic examination of the influence of season, and of maturity at the time of picking, upon pertinent aspects of the physiology of the fruit held at some appropriate storage temperature. The temperature adopted was 12.5° C. It was planned to combine with this survey such bulk storage tests as the results of the physiological enquiry might demand.

This project in its two aspects of physiological survey and storage testing is still in progress. The physiological work done by the writer has chiefly taken the form of tracing the respiration of the fruit throughout its life at the storage temperature. This choice was made because the record of respiration is the most generally useful single index of the gradually changing physiological state of the fruit. To be fully significant such a study demands the treatment of single fruits, otherwise individual peculiarities are obscured by statistical effects. This would defeat one main purpose of the enquiry, which is to detect and characterize at least the more frequent physiological types occurring amongst the fruits at all seasons of the year in correlation with the cyclic variation of the conditions of growth.

The present paper gives an account of the respiration at 12.5° C. of individual fruits picked at various stages of maturity from plants grown in the greenhouse at different seasons during the first year of the investigation. This systematic survey was continued for another two years with confirmatory results, so that a much larger number of fruits have actually been under observation than are reported upon in detail in this paper. It happens that the data of the first year, with slight supplementation, illustrate sufficiently the course of respiration in the fruits, and mere recapitulation of substantially similar results could serve no purpose. The individual records will be examined and compared in some detail, since they form an index to the physiological changes occurring in fruits stored at 12.5° C. They are also the criteria by which we distinguish two types of fruit, the distribution of which has been found to be correlated with the seasonal factor. These types will be constantly before us in later papers of this series, which will embody the results of attempts to modify the distribution of the types through the manipulation of growth conditions, the further characterization of the types, and the history in bulk storage of fruits produced by various methods.

Experimental Procedure

Propagation of the Plants and Growth of the Fruit

Tomato plants of Grand Rapids variety were grown in the greenhouse from seed obtained from a commercial seed house. A standard procedure for germination and transplanting was devised, and the soil kept as uniform as possible throughout. The bench soil was a light clay loam with which was incorporated well rotted manure (30 tons per acre) and a 0-12-12 fertilizer (1500 lb. per acre). When the third truss of fruit was forming, weekly side dressings of nitrate of soda were applied (150 to 200 lb. per acre). The plants were set out with 18-inch centres and grown to a single stem. They were supported and allowed to produce seven trusses of fruit.

The mean temperature of the greenhouse varied with the season, but abrupt or violent fluctuation was avoided. The light periodicity normal to the season was not modified in any of the experiments dealt with in this paper. Pests were not serious, but some fumigation was unavoidable and cyanide was employed at least two weeks before picking the fruit. There was no detrimental effect and we are satisfied that any physiological disturbance occasioned at the time of fumigation was well past before respiration experiments began.

Pollination was assisted by tapping the flowers. The setting of the fruit was identified by the ready separation of the corolla from the receptacle and the date of setting was recorded on a tag attached to each young fruit.

Classification of the Fruits

The age of each fruit was known but the growth and physiological development of the individuals vary, so that fruits of the same chronological age are not necessarily in identical physiological states. It was essential, therefore, to consider other indices of development in choosing the experimental fruits. Consequently, a series of classes was established, within the limits of each of which the fruits would be at least roughly comparable physiologically. This classification is given in Table I. The subsequent physiological test confirmed the assignment of fruits to their place in this classification in all but exceptional cases.

Measurement of the Respiratory Carbon Dioxide

Immediately after picking, the fruits were removed to a room at 12.5° C. the calyces were carefully taken off, the fruits weighed, the calyx scars waxed and the fruits re-weighed. A full description of each fruit was recorded. The fruits were then placed singly in glass respiration chambers consisting of two hemispheres sealed together and provided with tubulatures. The air flow was started, and zero time of the respiration record was taken the following morning, approximately 15 hours later. The air was passed through large towers of soda lime and humidified in 7% potash solution before being led into the respiration chambers. From the chambers each stream passed to a Pettenkofer absorption tube containing barium hydroxide. The Pettenkofer tubes were changed periodically and the residual baryta titrated. The

TABLE I

PHYSIOLOGICAL CLASSIFICATION OF TOMATO FRUITS

Physiological stage	Symbol indicating physiological state on respiration records, tables, etc.	Description of the fruit
Early growing-green	I	Immature, growing, hard, dark green and small in size for the variety. Chronological age up to 32 days.
Late growing-green	II	Maturing, hard, green, medium size for the variety. Chronologically 32 to 42 days.
Mature-green	III	Maximum growth, firm, light-green color.
Yellowing	IV	Yellow color appearing on the base, remainder light green.
Yellow-orange	V	Orange-red base and yellow-green calyx.
Three-quarters red	VI	Evenly orange-red.
Full red	VII	Red.

successive absorption periods were of 24 hours duration in the experiments on fruits from Populations I, II and III, and of 12 hours in the others. Mean rates for each interval were computed in terms of cc. of CO_2 per 10 kg. of fruit per hour. These determinations are represented graphically in Figs. 1 to 35.

As the experiment proceeded, notes were kept of the maturation and senescent changes in the fruit. Where the fruit cannot be handled or destroyed, the only available index of ripeness is external color, so that the correlation of respiration rate with ripeness is essentially a correlation with color. Each graph is provided with a chart which indicates the progress of external color change along the time scale of the respiration record.

The Experimental Materials

The fruits used in the survey experiments of the first year were derived from four plantings of tomatoes which are referred to as Populations I to IV. They were picked at various stages of maturity, but owing to limitations of apparatus, each population could not be represented by fruits of every stage. The character of the fruits is indicated in Table II, in which they are assigned to their parent population and classified according to the scheme previously described.

Figs. 37 and 38 summarize the chronological distribution of the periods of growth and of picking for each population and relate them to the factors of light and temperature.

Populations I and II were summer-grown, and since the records indicate that the fruits of the two populations constitute a single series, they are

TABLE II

NUMBERS OF FRUITS OF POPULATIONS I TO IV, IN VARIOUS PHYSIOLOGICAL STAGES

Population	Early growing-green	Late growing-green	Mature-green	Yellowing	Yellow-orange	Three-quarters red	Full red
	I	II	III	IV	V	VI	VII
I			2	2	2	2	3
II	4	3					
III			3	2		3	2
IV	3				2		2

treated together. Population III was seeded in July and the fruits examined were autumn-grown. Certain of these fruits provided respiration records that form a striking contrast with corresponding summer-grown fruit. The implication of the respiration studies is that the vast majority of autumn-winter-grown fruits when isolated at appropriate stages of maturity and kept individually at 12.5° C. are in a different physiological state from the summer-grown fruit. The difference is interesting from the point of view of storage because it involves a great extension of life at the temperature employed. The plants of Population IV were started in winter, but fruited in spring and represent the spring population.

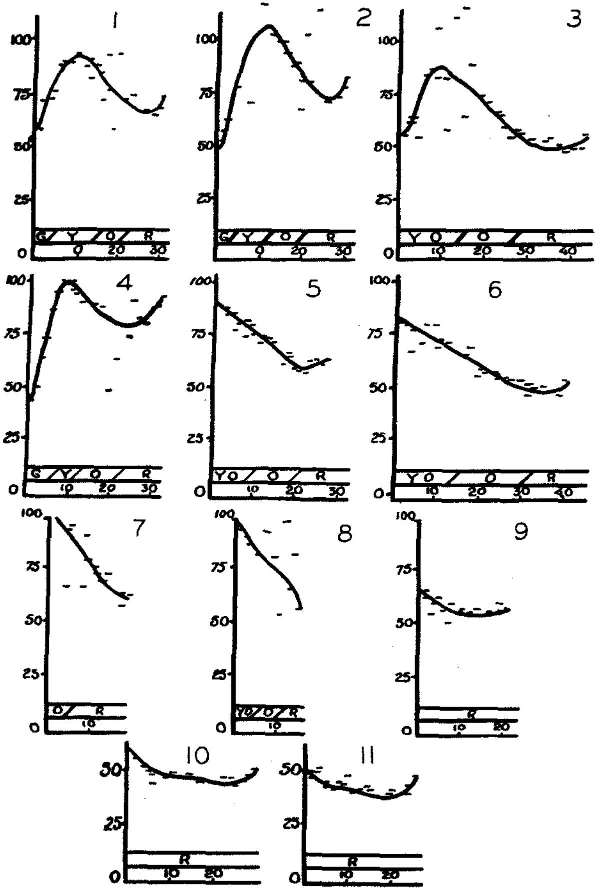
Experimental Results

Fruits of Populations I and II

The respiration records of eleven fruits from Population I are given in graphic form in Figs. 1 to 11. Before turning to the data, comment must be made on certain fluctuations of great amplitude occurring in some of the earliest records obtained. These fluctuations are quite distinct from the normal fluctuations found in every respiration record. Their origin was traced to stoppage of the air stream by deposition of barium carbonate in the narrow delivery tubes with which the Pettenkofer tubes were originally furnished. Replacement of the delivery tubes removed the difficulty. Points known to be affected by this circumstance are neglected in drawing the lines.

Records 1 and 2 are of fruit classified as Stage III, mature-green at the time of picking. Respiration rises steadily to a maximum of 100 to 110 cc. CO_2 in 12 days and then drops to about 70 cc., completing the rise and fall in about 27 days. After a few days of steady rate tissue breakdown occurs, carbon dioxide output rises, and the determinations end.

The ripening color changes commence almost immediately after the beginning of the record, so we must conclude that the fruits were in the last stages of mature greenness at the time of picking. The fruits change from yellow-green to orange during the rise of respiration and the transition to red is



FIGS. 1–11. *The respiratory drift in storage (at 12.5° C.) of fruits of Population I. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing YO = yellow-orange, O = orange, R = red).*

accompanied by falling respiration rates. The correlation of the visible ripening color changes with a rise and fall of respiration confirms Gustafson (4), who found that ripening tomatoes pass through these respiratory phases. This phenomenon appears to be an almost general property of senescent plant tissues. The "senescent rise of respiration" was long ago observed by Blackman (2) in starving leaves, and more recently by Blackman and Parija (3), Kidd and West (5, 6, 7) and others, in isolated fruits such as apples and pears. It must be regarded as the conventional mode of behavior of ripening fruits, departures from which should be noteworthy.

The ready identification of the form of these records with the conventional senescent phases of respiration makes it clear that the records are devoid of initial phases which would indicate a change of physiological state as an immediate result of isolation or cooling. Hence the initial rates of the records may be taken as approximations to rates characteristic of the fruit before detachment from the plant. A series of such initial rates obtained for fruits isolated at various states of maturity ought, therefore, to indicate the manner in which the respiration is changing whilst the fruit is on the plant. This in turn should enable us to determine the relation between the course of respiration on the plant and the course after isolation at the storage temperature, as well as any changes which the seasonal factor may induce in this relation. The initial rates of these two fruits are of the order of 50–60 cc. CO_2 per kg.-hr.

Records 3 and 4 are of fruits which were picked just as the ripening color changes began, and at picking were classified as in Stage IV of maturity, very slightly in advance of Fruits 1 and 2. The respiration records are correspondingly similar and clearly represent the conventional senescent respiratory phases accompanying, in the same manner as before, the visible changes of color. The initial rates are also of the same order as those of Records 1 and 2.

The next pair of records (5 and 6) are of fruits picked in the yellow-orange or fifth stage of maturity. These records are quite different from the four that have just been examined. The initial rates are of the order of 90 cc. CO_2, which is approximately that of the peak values of Records 1 to 4, and the form of the records is that of a simple, gentle decline. In Fruit 6 breakdown occurred two weeks later than in Fruit 5. The change in color from yellow-orange, to orange, to red, accompanies these falling rates just as it accompanies the declining respiration phase in Records 1 to 4.

Judging from the initial rates of Records 5 and 6, respiration on the plant has increased substantially between Stages IV and V. We conclude, therefore, that Fruits 5 and 6 had already passed into the senescent rise of respiration before they were picked, that they were picked near to the peak of the rise and that their records of respiration after picking represent the declining arms of senescent phases which remain to be completed, on a modified time scale, at the low temperature.

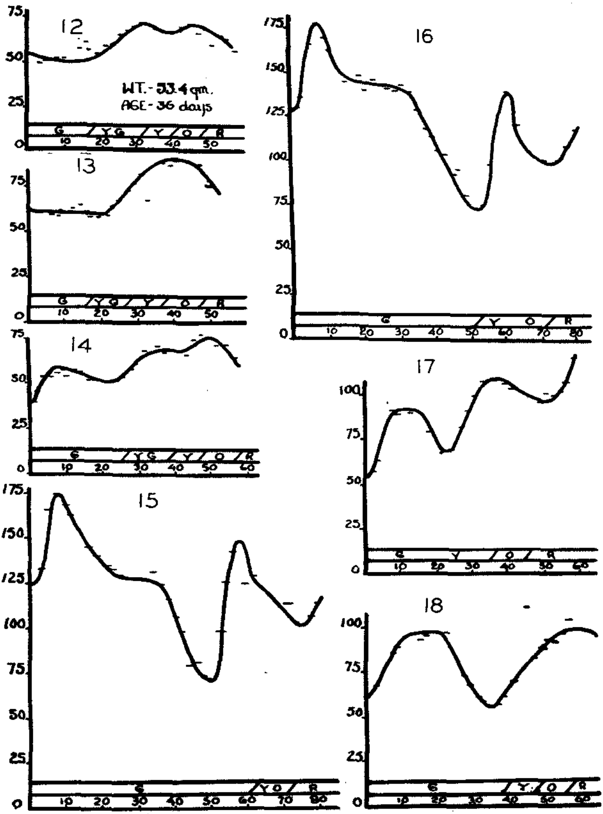
Stage VI fruit is represented by Numbers 7 and 8. The form of these records is again a decline, but this time the decline is steep. The initial rates of about 100 cc. CO_2 per hr. are very high, indicating probably a further increase over Fruits 5 and 6 of respiration while still on the plant. The conclusion suggested is that Fruits 7 and 8 were picked at or just after the maximum of the senescent rise on the plant and they then completed what remained of the decline after isolation at 12.5° C.

Records 9, 10 and 11 are of fruits which were full red at picking. If the indications of the other records have been properly interpreted, the larger part of the senescent rise and fall of respiration in these fruits should have been passed through on the plant and we should, therefore, expect that the terminal portions only would remain to be gone through in isolation. The form and pitch of Records 9, 10 and 11 obviously fulfil this expectation.

Seven growing-green fruits of Population II were examined, and their respiration records are numbers 12 to 18. The first three were picked at the end of the growing-green stage; the remaining individuals were much less advanced in that stage when they were removed from the plant.

Records 12 to 14 begin with a more or less steady or slightly declining phase, introduced in Record 14 by an initial rise. The steady phase continues with more or less random fluctuation for 20 to 25 days, after which the rate of respiration goes up. This increase in rate is correlated in each record with the external ripening color changes in the fruit, hence we must conclude that the rise and subsequent decline represent the senescent rise of respiration. These records differ in form from Records 1 to 4 (mature-green fruit) essentially in the presence of the extended steady phase antecedent to the senescent rise. The presence of the steady phase suggests that the fruit passes through a similar phase while on the plant. If so, then Fruits 12 to 14 must have been picked well before this phase was past, so that they continued in it for 20 days after isolation before passing into the senescent phase. Fruits 1 and 2, on the other hand, must have been picked after this phase was over, for they are in the senescent rise from the beginning of the record. Moreover, the initial rates of Fruits 1 and 2 are of the same order as those of 12, 13 and 14, *i.e.*, 50 to 60 cc. CO_2, so that whatever interval of development on the plant separates the two groups of fruits, it must be an interval of relatively steady respiration.

If Fruits 12, 13 and 14 were picked just as they reached the border line between late growing- and mature-green, Fruits 15 and 16 were certainly remote from the border line and were in fact the youngest fruits to have been examined in any of the populations. Fruits 17 and 18, though assigned with Numbers 15 and 16 to the physiological Class I (early growing-green) were considerably farther advanced than the latter. They were taken from the field to supplement the fruits of Population II because at the time of the experiment, the greenhouse crop did not afford examples of fruit at precisely the right stage.



FIGS. 12–18. *The respiratory drift in storage (at 12.5° C.) of fruits of Population II. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

Dealing first with the physiologically younger pair of fruits, Numbers 15 and 16, inspection of the respiration record reveals an extremely complex form, yet one which is evidently characteristic of the state at the time of isolation, since the two records correspond in every detail. The records start off with initial values of about 130 cc. CO_2. and for the first week respiration rises until a maximum of about 180 cc. CO_2 is reached. Then a fall sets in, which is interrupted by a temporary steady state lasting for about 10 days, and then resumed until a transitional minimum of about 70 cc. is reached after 50 days. The minimum gives way at once to a sharp rise which is correlated in time with the first visible ripening color changes and is, therefore, to be identified as the senescent rise. This soon reaches a maximum and the records terminate in the manner of Records 1 to 4.

For our present purpose it is not necessary to enter more fully into the characters of these records and their physiological significance. They seem to be special cases of the general form which also underlies the respiration records of starving leaves and immature apples. In conformity with this, the initial rates of 130 cc. CO_2 are very high, indicating an altogether higher order of respiratory rate in the young growing fruit on the plant than at any subsequent stage of development.

Records 17 and 18 begin with an initial rise similar to that found in Records 15 and 16 and also in Record 14. Since this cannot be the senescent rise, it must have some other significance. Experience shows that this initial effect is characteristic of fruits isolated in the early stages of development when starch is abundant. As starch diminishes, this feature disappears from the records. Amongst the present data it is found in the records of all the very starchy fruits, in one out of three of the fruits which are rapidly losing their starch, and in no others. When the initial effect is over, respiration is found to be in a steady state similar to that which interrupts the declining phase in Records 15 and 16. After the steady phase the second limb of the declining phase appears, followed by a transitional minimum and the senescent rise. The form of Records 17 and 18 is therefore that of Records 15 and 16 with the first half of the declining phase omitted. The initial rates are of a very much lower order, suggesting that the interval separating the stage represented by Fruits 15 and 16 from that represented by Fruits 17 and 18 is characterized by a very rapid fall of respiration in the fruit on the plant.

We may now consider the whole series of summer-grown fruits. Fig. 36 is a somewhat schematized plotting of the initial rates of the isolated fruits against time. This graph represents approximately the sequence of changes in respiration rate which characterizes the growth and development of the summer-grown fruit on the plant. When we compare the series of respiration records given by isolated fruits with this graph and with each other, it is clear that the isolated fruit continues in modified form at the storage temperature the sequence begun on the plant, and does not recapitulate phases that have already been passed through. The records of fruits picked in later stages of maturity may therefore be regarded as derived from those of fruits

picked in earlier stages, by progressive omission of the early phases of the record. The metabolic history at the storage temperature is therefore determined by the point reached in the normal sequence at the time of picking.

The normal sequence of the tomato fruit growing and maturing on the plant is of the same form as that made familiar by extensive investigations on the apple and pear, and the physiological changes underlying it are presumably of general significance. The rule that the isolated fruit continues the sequence (in modified form) seems also to apply to other fruits, so that the summer-grown tomato gives no evidence of unconventionality in these respects. It is, therefore, interesting to find that autumn-winter-grown tomatoes, isolated in certain stages of development, appear to depart from this rule in a manner and to an extent that seems to have significance certainly for the scientific and possibly also for the practical aspects of the problem of preserving the fruit in the living state.

Fruits of Population III

The anomalous behavior of fruit from this as well as from subsequent autumn-winter populations of plants draws attention to the seasonal differences in growth conditions with which this variation appears to be correlated. The components of the seasonal factor for which some records are available are temperature and illumination.

The records of temperature in the greenhouse are incomplete. But fortunately it is not difficult to chart the course of mean temperature during the missing portions of the records from experience with sufficient certainty for the present purposes. In Fig. 37 the weekly mean maxima and minima are given separately for day and night. With the temperature chart is another which shows the whole growth period of each population and the interval of time during which the fruits were sampled. A similar set of charts for weekly mean hours of light and weekly mean hours of sunlight in relation to growth and sampling is given in Fig. 38. From these figures it is possible to form some conception of the manner in which these components of the seasonal factor varied from one population to another.

It is especially clear in connection with illumination that the autumn-winter population was grown under conditions that were radically different from those obtaining during the growth and sampling of the others. The summer populations were grown when this factor was rising from the minimum, and the fruit was sampled when it was maximal. The winter-spring population was grown when illumination was at first minimal, then rising, but the fruit was sampled when illumination was about half maximal. Population III on the other hand, was grown when illumination was falling from the maximum and the fruit was sampled at the minimum of this factor. There can be little doubt that in respect of light periodicity, intensity and quality, the autumn-winter period differs sharply from the summer periods, while the spring period is intermediate, but appears to have more affinity with the summer than with the autumn.

Mean temperatures tend to be steady and moderate from October to April, but reach high values in the other months of the year. In respect of temperature the autumn and winter-spring periods differ chiefly in the higher temperatures prevailing during the early growth of the autumn plants. The larger part of the two growth periods and both sampling periods were characterized by steady moderate temperature. The larger part of the growth period of the summer populations and their sampling periods were characterized by high maximum temperatures.

As a whole, the temperature and illumination components of the seasonal factor indicate a sharp difference between the summer and the autumn-winter periods, with the winter-spring period partaking of the characters of both. It might be supposed, therefore, that any influence season might have upon the physiological state of the fruit would be chiefly manifested in differences between fruits of summer and autumn-winter populations, with winter-spring fruits in an intermediate position. Owing to the fact that fruits of Stages III and IV are not represented in the winter-spring sample of the first year's work, it cannot be decided from the present body of data alone whether fruits of the autumn-winter type are found amongst the winter-spring population. But subsequent experiments show that the winter type does extend into the early spring. The transition from the winter to the summer type appears to occur in March to April.

We have little doubt that the physiological composition of the fruits of the several populations is a correlative of season. But the effect of the seasonal factor may be to influence the distribution of types which are actually present in all seasons rather than to evoke in one season a type quite unknown in another. Consequently to avoid confusing this issue until more is known we shall refer to the summer and winter types merely as the "conventional" and "anomalous" types respectively.

TABLE III

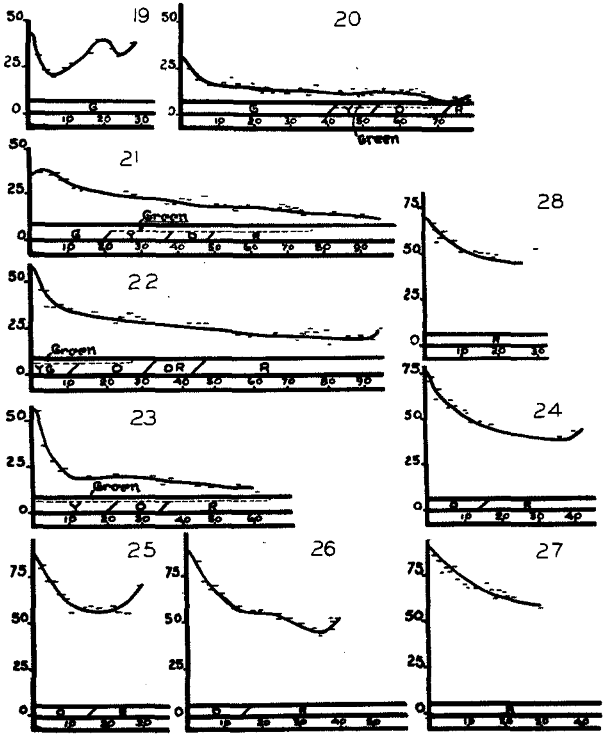
THE RELATION BETWEEN AGE AND PHYSIOLOGICAL STATE IN POPULATION III

Fruit No.	20	21	22	23	24	25	26	27	28
Stage	III	III	IV	IV	VI	VI	VI	VII	VII
Age	47	54	62	53	52	52	54	52	54
Growth interval	Oct. 3 Nov. 19	Sept. 26 Nov. 19	Sept. 28 Nov. 19	Oct. 9 Dec. 1	Sept. 28 Nov. 19	Oct. 10 Dec. 1	Sept. 26 Nov. 19	Sept. 28 Nov. 19	Sept. 26 Nov. 19

Table III gives for each fruit of Population III the stage of maturity at which it was picked, its chronological age and growth interval. The growth intervals vary only from 47 to 62 days and all but two lie between 52 and 54 days, yet the maturity stages represented lie between mature-green (III) and full red (VII). This sort of physiological heterogeneity amongst fruits of the same chronological age is found in other populations also but not to

the same extent as in this population. It will be shown later that the heterogeneity within a single fruit is also more marked in the fruit of this than in fruit of the other populations.

The respiration records are given in Figs. 19–28. The first of these, Number 19, is of a fruit which was classified at picking as on the border of growing-green and mature-green. A premature tissue breakdown associated with rising respiration developed in this fruit. Nothing of the sort occurred



FIGS. 19–28. *The respiratory drift in storage (at 12.5° C.) of fruits of Population III. Vertical axis indicates cc.* CO_2 *per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

in any other fruit of this population. The breakdown did not appear to have an infective origin, but there is no critical evidence that this is so. It might be characteristic of an extreme variant in a physiologically heterogeneous group. It is best, therefore, to reserve comment on this individual.

Fruits 20 and 21 were picked in the mature-green stage. The respiration records are low in pitch and the initial rates are of the order of 35 to 40 cc. CO_2. Record 21 gives slight indication of an initial rise such as characterized the earlier stages of corresponding fruits in Population II. From the initial value the rate drops more or less gently to a prolonged phase of low, almost steady, slightly declining respiration. This phase is of remarkable duration. The experiments had to be terminated before it came to an end and before the fruits showed the slightest sign of breakdown. The records, though very long, are nevertheless incomplete. In fruit of later populations records of this type have been observed which went on for 130 days.

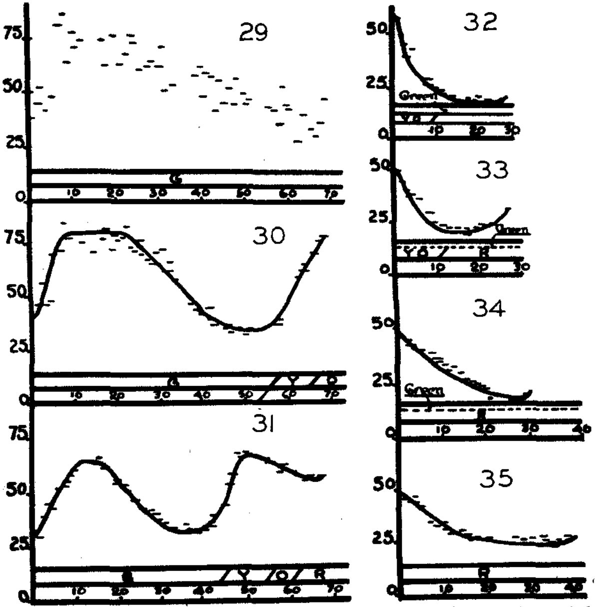
The only other fruits to pass through a long steady phase of anything like this appearance were Numbers 12, 13 and 14 of Population II. But in the latter, the steady phase passed into the senescent rise when the external color changes began, whereas in the present fruits the steady phase continues quite unbroken throughout the period of color change and long past the point at which the fruits had become full, ripe red. The only unusual feature of the ripening color changes was a marked tendency of an island of tissue in the immediate vicinity of the calyx to lag behind the rest of the fruit. Ultimately ripening was complete. The persistence of the calyx end indicates, however, that these fruits are characterized by a higher order of cell heterogeneity than summer-grown fruits.

It is evident that the steady phases of Records 12, 13 and 14 cannot be homologized with those of Records 20 and 21. Indeed the present records have little in common with those of summer-grown fruit picked at any stage whatever. They represent the anomalous type, the characteristics of which are a decline sometimes prefaced by an "initial effect" passing into a phase of steady respiration greatly extended in time, during the unbroken course of which the ripening color changes occur. The essence of the anomaly is the absence of the senescent rise, a phenomenon occurring regularly in relation to ripening not only in our summer-grown fruit but also observed as the rule for tomatoes by Gustafson (4). This type of record appears to indicate a state of physiological stability quite unusual for tomatoes and is associated with a duration of life at 12.5° C. several times as great as that characterizing corresponding fruits of the conventional type.

The next two fruits (Records 22 and 23) were isolated at Stage IV, just as they were commencing to turn yellow. The records are of the same general form as Numbers 20 and 21. The initial rates of 57 and 60 cc. CO_2 are higher than those of Records 20 and 21, from which it appears that while yet on the plant the fruits actually pass into the usual rising phase of respiration as the ripening color changes supervene. In this they resemble the conventional type. But the remarkable thing is that the yellowing fruits of the present

population, after they are picked, do not proceed with the senescent rise in the manner of the conventional type (Records 1 to 4). On the contrary, the records decline from the beginning, passing into an extended phase of precisely the character of that in Records 20 and 21. The ripening color changes also occur in the absence of a senescent rise, and the extraordinary duration of life which accompanied the stable state of Numbers 20 and 21 occurs in the present pair as well.

This series of records does not happen to include examples of fruits isolated in Stage V. Records 24 to 28 are of fruits picked in the orange (VI) and red (VII) stages of maturity. The first three were orange when picked. In the fruit of the summer populations this color, whether developed on the plant or after picking, at 12.5° C. is associated with the peak values of the senescent rise of respiration. This appears to be true of the attached autumn-winter



FIGS. 29–35. *The respiratory drift in storage (at 12.5° C.) of Population IV. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

fruits also for we find the initial rates of Records 24 to 26 in the vicinity of 80 to 90 cc. CO_2. The form of the records is that of fruits of corresponding maturity in the summer populations and, as before, evidently represents the declining arm of the senescent phases. No stable state is established and in 25 to 40 days breakdown occurs.

Of the two fruits picked full red, Number 27 has a high initial rate and a record very much the same in form as those of fruits that were orange when picked. Presumably it was actually on the border line of orange and red. The record of the other ripe fruit has a lower initial rate and in all its characteristics is indistinguishable from the corresponding ripe fruits of Population I. (Records 9 and 10). It would seem, therefore, that the stable characteristics which distinguish the isolated unripe fruits of autumn-winter populations from the corresponding summer-grown fruits are not manifested in the autumn-winter fruit if it is permitted to pass a certain point of maturity before it is picked. The evidence is that this point is roughly marked by the external appearance of red pigmentation in the fruit.

Fruits of Population IV

This is the winter-spring population and the records of respiration of the fruits studied are given in Figs. 29–35. The first of these is characterized by an unusual scatter of points referable to error in the measurement of the low

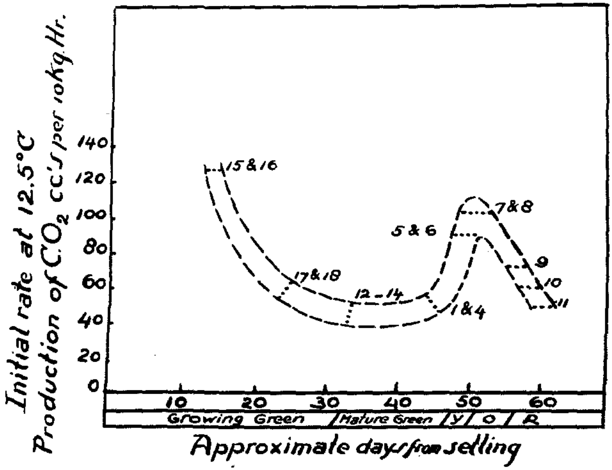


FIG. 36. *The drift of respiration of tomato fruits of Populations I and II on the plant as determined from the initial rates of the stored fruits of the summer-grown populations.*

CO_2 output of the very small fruit. There is evidently an "initial effect" such as appears in the records of all very young fruits, and thereafter a decline, but all details of form are obscured. The two fellow records (30 and 31) are of fruits not so early in the growing-green stage and their form is evidently that of Records 17 and 18, Population II, but they are of lower pitch. We conclude that winter-spring fruit isolated in March at the growing-green stage is physiologically comparable to corresponding summer fruit.

The next stages of maturity were not represented in fruits of this population, but the examination of subsequent winter-spring fruits indicates that the anomalous type appears, provided the fruits are picked early in the spring as well as at the appropriate stage of maturity. Later, the conventional type predominates.

Fruits 32 and 33 were picked just as the red pigment began to appear superficially. Their relatively high initial rates indicate that the senescent rise had set in while they were still on the plant. The records conform to the conventional type and are comparable to Records 5 and 6 of Population I except again for their lower pitch. Similarly Records 34 and 35 of fruits picked full red are comparable to Records 9, 10 and 11 of Population I.

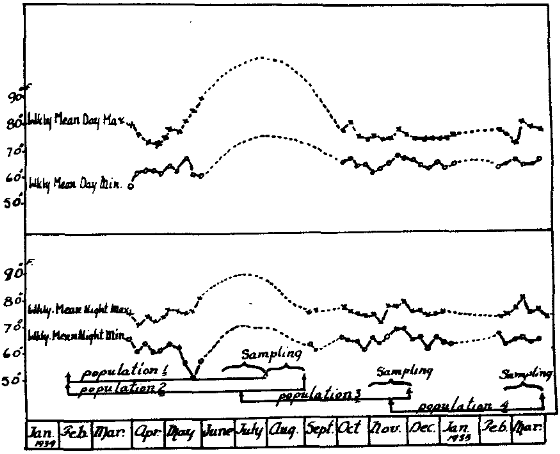


FIG. 37. *Temperature records in the greenhouse during the growth of Populations I to IV, showing the weekly mean day maximum, the weekly mean day minimum, the weekly mean night maximum and the weekly mean night minimum. These temperature records are not complete but are connected by dotted lines which indicate the probable temperatures approximating those obtained from outside temperatures. The period of growth and the sampling period for each population are indicated at the bottom of the figure.*

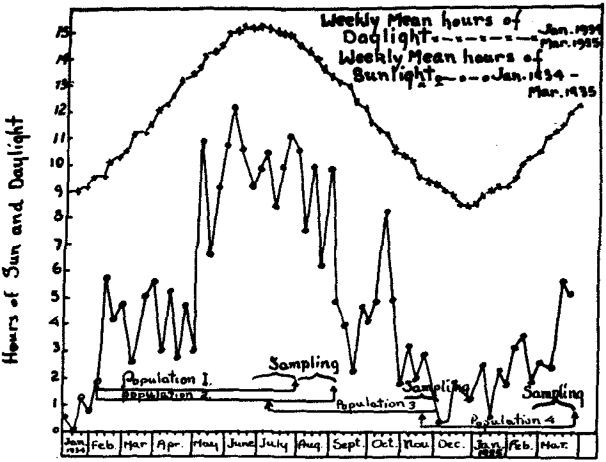


FIG. 38. *The weekly mean hours of daylight and the weekly mean hours of sunlight, together with the growing period and the sampling period for each of the four populations.*

The evidence provided by the data examined in detail in this paper, as well as of those for subsequent populations of tomatoes, indicates that in the course of ripening while on the plant, fruits pass through the senescent rise of respiration irrespective of the time of year in which they are grown in the greenhouse. During the late spring and summer the ripening processes of isolated fruit at 12.5° C. are also accompanied by the senescent rise of respiration in the conventional manner and are characterized by the lack of durability normal to this fruit. At other times of the year the course of metabolism in the fruit isolated at 12.5° C. depends upon the stage of maturity at which it is picked. If ripeness is approaching, as indicated by the external appearance of red pigment, the course of metabolism conforms to the conventional type and the fruit is not durable. But the autumn-winter-early-spring fruit, if picked approximately mature-green, departs from the conventional type. The respiration record reveals an extended period of remarkably steady rate while ripening is in progress and for long afterwards, suggesting a metabolically stable condition quite different from that found in fruit of the conventional type. This anomalous type of ripening is associated with a very much greater durability of the fruit both at 12.5° C. and afterwards, if removed to a higher temperature. The quality of such fruit in respect of texture and flavor when ripe is quite satisfactory.

Acknowledgments

The author is indebted to Mr. W. Hugh Smith, at whose suggestion this work was begun and to Dr. J. H. L. Truscott for guidance during the greater part of the time it was in progress. Professor G. H. Duff of the University of Toronto made many valuable suggestions in the formulation of the data and in criticism of the manuscript. To those gentlemen the author offers his thanks.

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The "BLACK-POINT" OR "KERNEL SMUDGE" DISEASE OF CEREALS[1]

By J. E. Machacek[2] and F. J. Greaney[3]

Abstract

A seed disease of wheat, rye, and barley, characterized by a more or less conspicuous brown or black discoloration of the kernel, particularly in the region of the embryo, frequently reduces the seed value and usually the sales value of affected grain. A comprehensive review of the relevant literature is given. The name "kernel smudge" is suggested for this disease. Two species of *Alternaria* (*A. tenuis* and *A. Peglioni*) and two of *Helminthosporium* (*H. sativum* and *H. teres*) are the fungi chiefly associated with it in Manitoba. The kernel smudge caused by *Alternaria* cannot be accurately distinguished from that caused by *Helminthosporium*, without a laboratory examination of the seed.

Extensive trials with wheat have demonstrated that the *Alternaria* type of kernel smudge does not affect to any marked extent seed germination, plant emergence, intensity of root rot, and yield in the subsequent crop. It was established, however, that *H. sativum* does reduce germination, seedling emergence, and the yield, and at the same time causes an increased amount of root rot in this crop.

Under Manitoba conditions infection of the kernels arises from air-borne spores which are usually deposited in the largest numbers at about the time the kernels are maturing. Contrary to some observations made elsewhere, the disease does not result in shrunken kernels; the largest kernels are frequently infected, and the small, shrunken ones usually free from the disease. Apparently the reason for this is that the large kernels force open their covering glumes, thus affording access to air-borne spores, whereas the glumes of small kernels remain closed and exclude such spores.

The seed value of grain attacked by the virulent (*H. sativum*) type of kernel smudge was increased considerably when such grain was dusted with suitable organic mercury dusts (ethyl mercury phosphate or methyl mercury nitrate). Dusting with copper carbonate was relatively ineffective. The development of kernel smudge in the maturing crop was not prevented by dusting the growing plants with sulphur.

Introduction

A disease of cereals, frequently referred to as "black-point", occurs in most grain-growing areas of the world and causes considerable loss. The literature relating to it is fragmentary and not infrequently conflicting: the exact nature and cause of the disease are not clearly defined. In this paper a general review of the results obtained by other workers is given, and an attempt is made to discuss different aspects of this disease in the light of observations and experiments made in Manitoba during the eight-year period, 1929 to 1936.

Economic Importance

This disease has been reported as present in Canada, the United States of America, Argentina, Germany, Italy, Morocco, South Africa, India, and Java, and probably it occurs wherever cereals are grown. It attacks wheat, rye, and barley, as well as certain cultivated and wild grasses. As yet, however, few investigators have attempted to estimate the loss resulting from

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Contribution No. 515 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
[2] *Assistant Plant Pathologist, Dominion Rust Research Laboratory, Winnipeg, Manitoba.*
[3] *Plant Pathologist, Dominion Rust Research Laboratory, Winnipeg, Manitoba.*

this pathological condition of the seed. Bolley (5), in referring to the damage caused by ***Helminthosporium sativum*** to wheat grown in North Dakota during 1920, stated that this organism was very abundant and particularly destructive "in destroying stools and in the production of black points in the grain". According to Weniger (52), black-point of wheat caused a loss of 0.5% in North Dakota in 1925, but it was less severe that year than usual.

An idea of the prevalence of this disease in Manitoba can be gained from Table I in which is given the percentage of discolored kernels found in eight varieties belonging to the wheat groups *Triticum durum* and *T. vulgare*. These varieties were grown at Winnipeg during the seven years, 1929 to 1935. It would appear from this table that the *durum* wheat varieties are more susceptible to this disease than are the *vulgare* varieties. This relation was most noticeable in 1935.

TABLE I

THE PREVALENCE OF DISCOLORED KERNELS IN THE THRESHED GRAIN OF WHEAT VARIETIES GROWN AT WINNIPEG, MAN., DURING THE 7-YEAR PERIOD, 1929–1935. (PERCENTAGE OF KERNELS DISCOLORED IN COUNTS OF 2,000)

Variety	1929	1930	1931	1932	1933	1934	1935	Mean
T. durum								
Acme	1.3	22.2	11.0	42.8	30.8	69.0	65.3	34.6
Pentad	0.8	—	21.7	37.3	40.7	63.7	93.6	42.9
Mindum	2.6	11.5	15.7	—	—	24.2	55.0	21.8
T. vulgare								
Marquis	0.4	4.1	2.6	—	0.7	—	10.8	3.7
Reward	0.0	—	0.2	—	0.7	—	4.0	1.2
Ceres	—	—	0.0	—	1.8	—	5.3	3.5
Pentad × Marquis (R.L. 723)	—	5.8	27.6	2.4	—	—	—	11.9
Pentad × Marquis (R.L. 726)	—	—	18.3	2.3	0.7	5.4	—	6.7

The presence of discolored kernels in the threshed grain lowers the market value of a crop. To command the highest price, grain, whether wheat for milling or barley for malting, should be uniformly bright in color. Grain merchants usually associate poor color with poor quality of the grain. Millers of durum wheats have a particular reason for avoiding grain containing a large proportion of discolored kernels. They find it exceedingly difficult to remove the dark specks which appear in the semolina milled from such grain, and when these specks appear in the finished products, macaroni, vermicelli, etc., the quality of the product is appreciably reduced. Furthermore, discoloration of the seed indicates a diseased condition and often the presence of parasitic fungi that may attack and destroy the seedling plants if seed thus affected is sown.

Nomenclature

"Black-point" was a term first used in 1913 by Bolley (5) to describe a wheat-kernel discoloration supposedly due to fungal invasion. Previous to this date, however, Zöbl (53) in 1892, and Puchner (35) in 1897, used the

adjectives "braunspitzige" and "schwarzspitzige", respectively, to describe analogous symptoms in barley. Ravn (36) followed the nomenclature of Zöbl. Coons (11), in 1918, used the term "black point" for a disease of wheat in which the main symptom was a blackening of the kernel tips. He found a bacterium, tentatively identified as *Bacterium viridi-lividum*, in the discolored tissues. Louise Stakman (45) isolated a fungus which she considered to be *Helminthosporium sativum* P., K., & B. from discolored kernels of wheat and rye. She stated that the disease symptoms produced by this fungus on the kernel differed distinctly from those of the so-called "black tip". The "black tip" disease referred to here is presumably that caused by the basal glume-rot organism, *Bacterium atrofaciens* McCulloch. Weniger (51) and Evans (15) found that *H. sativum* was usually associated with a kernel-discoloring disease of wheat which they called "black point"

Drechsler (14) showed that while both *H. sativum* and certain unidentified species of *Alternaria* were associated with the blackening of wheat kernels, the disease symptoms produced by these two types of organisms were slightly different. He retained the nomenclature used by Weniger and Evans. Rosella (37) and Miège (29) designated the kernel-blackening disease as "Moucheture". Peyronel (34) reported that in Italy the disease was known as "Puntatura". Pasinetti (33), describing a disease of San Martin wheat in which the kernels became blackened as the result of some unknown physiological derangement within the plant, stated that the name "Punta Nera" was applied to this disease by the Argentine growers. Dastur (12) showed that *H. sativum* was the principal cause of "black point" in wheat grown in India. Simmonds (41) applied the name "smudge" to wheat-kernel discolorations caused by *H. sativum*. Mead (27) used the same name for a similar disease in barley. In a more recent paper, Christensen and Stakman (10) refer to barley-kernel discolorations caused by *H. sativum* and species of *Alternaria* and *Fusarium* as "seed blight".

For many years bacterial and fungal discolorations of wheat, barley, and rye have been commonly termed "black point". In recent years, however, different workers, by observation and experiment, have established that definite and important differences exist between these two types of seed discolorations. The term "black-tip", referred to by Louise Stakman (45), aptly describes the bacterial type of kernel discoloration, while the term "smudge", as employed by Simmonds (41), more adequately describes the symptoms produced by fungal infection. The term "smudge" does not, however, indicate the location of the disease on the host plant. It is suggested, therefore, that the name "kernel smudge" be applied to the disease of wheat, rye, and barley, in which a dark or pale-brown diffuse discoloration occurs on any part of the kernel, particularly in the region of the embryo, and which is produced by fungi, especially those belonging to the arbitrary group Moniliales–Dematiaceae of the Fungi Imperfecti. Wheat kernels attacked by "kernel smudge" are illustrated in Plate I, F and G.

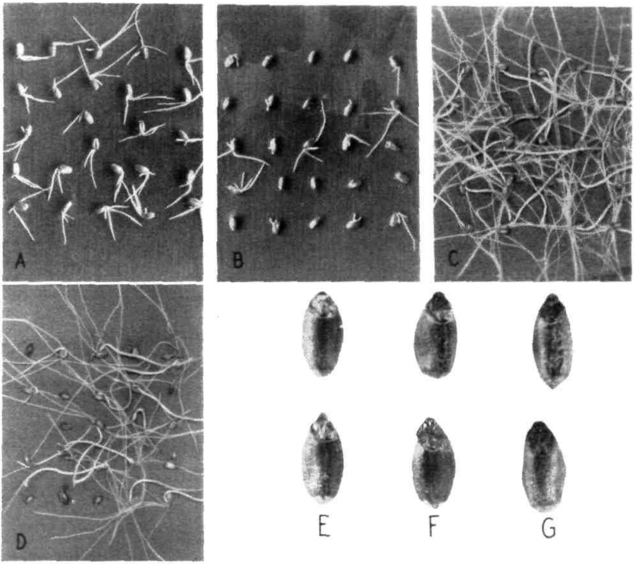

PLATE I. *A-D. The effect on germination and growth in Pentad wheat of two types of kernel smudge. A and C, kernel smudge (Alternaria type) of 1934 crop. B and D, kernel smudge (Helminthosporium sativum type) of 1935 crop. A and B, growth after 3 days. C and D, growth after 6 days. E-G. Kernel smudge on kernels of Pentad wheat. E, healthy kernels. F, discolored kernels (Alternaria type) of 1934 crop. G, discolored kernels (Helminthosporium sativum type) of 1935 crop.*

Etiology

Zöbl (53) isolated several different fungi from discolored barley kernels, but concluded that one of these fungi, *Cladosporium herbarum*, caused the discolorations. Peyronel (34), however, showed that while *C. herbarum* was sometimes associated with this disease it was not the cause. Ravn (36) found that *Helminthosporium teres* occurred in discolored barley kernels. Bolley (5) concluded that species of *Alternaria* and *Helminthosporium* were the chief causes of seed discolorations in wheat, although he found species of *Fusarium* in the diseased kernels as well. Hoffer (21) isolated *Fusarium* sp. and *Macrosporium* sp. from discolored wheat kernels. Palm (32) reported that, in Java, wheat kernels sometimes were infected by *H. gramineum*. According to Drechsler (14) the fungus isolated by Palm was not *H. gramineum* but *H. sativum*. Louise Stakman (45) isolated *H. sativum* from the discolored kernels of wheat and rye. Elsewhere (46) she reported that *Alternaria*, *Botrytis*, *Fusarium*, and *Tilachlidium* were found associated with the seeds of cereals. The strains of *Alternaria* which she isolated belonged to two general types. Evans (15) reported that 77.6% of the kernels in a sample of *durum* wheat were infected by *H. sativum*. Bassi (2) identified the causal pathogen of a seed disease of wheat as *H. gramineum*. This fungus, reported by Bassi as causing a conspicuous discoloration of the kernels, is believed by the writers to have been misnamed, being not *H. gramineum* but *H. teres*. The latter has been frequently isolated from discolored wheat kernels by the writers.

Christensen (8) found *H. sativum* in discolored seed of wheat, rye, and several grasses. Weniger (51) considered that *H. sativum* was the principal cause of kernel discoloration in wheat. Henry (19, 20) isolated a number of unrelated fungi from discolored wheat kernels, but *H. sativum* and a species of *Alternaria* were found most often. Drechsler (14) concluded that both *H. sativum* and *Alternaria* spp. caused seed discolorations in cereals. Rosella (37) found two species of *Alternaria*, *A. tenuis* and *A. Peglioni*, causing seed discolorations of wheat grown in Morocco, and (38) that *H. gramineum* caused a similar disease in barley. O'Gara (31) isolated *Podosporiella verticillata* from diseased wheat kernels. Dastur (12) showed that *H. sativum* was responsible for most of the discolorations of wheat seed produced in India. Waldron's (49) work suggested that wheat kernels were probably infected with *H. sativum* as frequently as with species of *Alternaria*. Simmonds (41) showed that *H. sativum* was a very common contaminant of seed wheat. Christensen and Stakman (10) isolated a number of different fungi from diseased barley kernels, but found that species of *Helminthosporium*, *Fusarium*, and *Alternaria* were present most often. Isolation studies made by Machacek (23), in 1925, indicated that *Alternaria* spp. were the fungi most commonly present in the kernels of wheat and rye grown in Saskatchewan, while *H. sativum* and species of *Fusarium* occurred much less frequently in these cereals.

During the five-year period, 1931 to 1935, the writers investigated the fungus flora in discolored kernels of wheat, barley, and rye. In the first four

years of this period the predominating fungi in such kernels were *Alternaria tenuis* Nees and *A. Peglioni* Curzi; the number of isolates of these two fungi exceeded by three times the total of the other fungi isolated. *Helminthosporium sativum* and *Fusarium* spp. were found only seldom. In 1935, however, most of the discolored wheat kernels in the samples examined were found to be infected with *H. sativum*, while *Alternaria* spp. were recovered much less frequently than in the previous years of the investigations. Occasionally, particularly in barley, exceptions were found. For instance, in 1934, a sample of discolored barley seed yielded several fungi in the following proportion: *H. teres*, 73.0%; *H. sativum*, 12.0%; *Alternaria* spp., 11.2%; *Fusarium* spp., 3.8%. In this sample, *H. teres* apparently caused most of the seed discoloration.

Methods of Isolation

A number of different techniques have been used by various workers to isolate fungi from discolored kernels. The most important difference in these techniques was in the method used to surface-sterilize the seed. Hoffer (21) sterilized wheat seed by dipping it in an alcoholic solution of mercuric bichloride. Norton and Chen (30) pre-soaked the seed for 10 to 12 hours in water and then sterilized it according to the method of Hoffer. Henry (20) did not pre-soak the seed, but first disinfected it with an alcoholic solution of mercuric bichloride, and then rinsed it with alcohol to remove the superfluous metallic salt adhering to the seed.

Although chemical treatments have been used extensively for disinfecting the seed of cereal crops, considerable criticism concerning their use for surface sterilization of infected kernels has been made. Louise Stakman (45) objected to the use of mercuric bichloride for seed which was infected with both *Helminthosporium sativum* and *Alternoria*. She found that *H. sativum* was more easily destroyed by this chemical than was *Alternaria*. Thus it would be presumed that, by this method of sterilization, *Alternaria* would be isolated more frequently than *H. sativum* from diseased seed, even when possibly more of the kernels were infected with *H. sativum* than with *Alternaria*. Mead (28) isolated from infected kernels, sterilized with silver nitrate, fungi with pale mycelium more frequently than fungi with dark mycelium, and thereby confirmed the finding just referred to, that fungi may respond differently to a single chemical.

To avoid any such selective action by chemicals in seed disinfection, attempts have been made to develop better methods for surface-sterilizing seed. Simmonds (41) removed surface-borne contaminants from seed by washing it in sterile water. In some cases he used a surface-tension-reducing substance in order to wet the seed thoroughly. He (42) constructed a device to wash seed or other plant parts. He was able to isolate a greater variety of fungi when he washed the diseased seed than when he disinfected it with chemical reagents. Atanasoff and Johnson (1) subjected barley seed infected with *H. sativum* to dry heat (95°–100° C. for 30 hr.) and found that this fungus was either inactivated or destroyed by the treatment, for the seedlings

arising from the treated seed were perfectly healthy. Mead (28), having investigated several methods of destroying or removing surface contaminants from the seed of cereals, concluded that the use of chemicals was satisfactory where completely sterile seed was desired, but that a more accurate determination of the fungus flora of seed could be obtained by the "Washer" method of Simmonds (42). He found that the use of dry or wet heat was unsatisfactory for purposes of seed sterilization.

As it is sometimes desirable to know the value of discolored grain for seed purposes, a practical and efficient method of diagnosing the type of seed discoloration is required. In an attempt to devise a satisfactory method whereby the seed value of large samples of grain could be determined, Simmonds and Mead (43) subjected a large number of samples to the following tests: (i) visual examination, (ii) centrifuge tests for the determination of surface-borne fungi, (iii) germination tests in sterile sand. In addition, the fungi within the seed were determined by the usual plating-out method, and, to some extent at least, by a microscopical examination of the kernel pericarp. The results indicated that the methods employed gave a very complete determination of the fungi within and on diseased kernels.

In the course of the present investigations, a simple, practical, and efficient method was used to determine the presence of fungi in seed affected by kernel smudge. This method is a modification of that used in the "plating-out" of diseased seed, the chief difference being that the surface-sterilized kernels are plated in soft agar, instead of on the surface of hardened agar, in Petri dishes. The kernels are first sterilized by being soaked for three minutes in ethyl alcohol-mercuric bichloride solution (one part 95% alcohol to three parts 1 : 1000 solution mercuric bichloride). While the seed is being disinfected, melted potato-dextrose agar is poured into sterile Petri dishes, and cooled to a temperature of about 60° C. The sterilized kernels are then planted in the soft agar, after which the agar is cooled as quickly as possible. Thus, the soft agar partially envelops the kernels, and the excess alcohol and mercuric bichloride on the surface of the kernels are absorbed or inactivated by the agar. In this process the seed is handled only once after undergoing sterilization, and hence the opportunity for it to become recontaminated by air-borne spores is greatly reduced. After a few days of incubation, the fungi within the diseased kernels emerge and grow on the surface of the agar, from where they can be readily isolated for further study.

The effectiveness of this method of isolating fungi from discolored seed was demonstrated during an examination of two samples of Pentad wheat, one from the 1934 and the other from the 1935 crop. A macroscopical examination of the seed showed that both samples were severely attacked by kernel smudge, there being little difference between the samples either in degree of discoloration or in the number of discolored kernels. Germination tests on moist blotting paper, however, indicated that there was a very marked difference in the ability of the two samples to germinate (Plate I, A-D). The 1934 seed germinated well, and although fungus mycelium emerged from the

TABLE II

Relative frequency with which *Helminthosporium sativum*, *Alternaria* spp., and *Fusarium* spp., were isolated from discolored kernels of Pentad wheat produced in Manitoba in 1934 and 1935

Crop year	Percentage of discolored kernels yielding		
	H. sativum	*Alternaria* spp.	*Fusarium* spp.
1934	1.9	81.4	0.00
1935	85.3	14.0	0.02

kernels, the seedling remained healthy for the entire experimental period of six days. On the other hand, the 1935 seed germinated very poorly, and many of the seedlings were badly diseased. By the modified plating-out method described above, 81.4% of the discolored kernels in the 1934 sample yielded *Alternaria*, while this fungus was isolated from only 14.0% of the 1935 seed. On the other hand, 85.3% of the discolored kernels in the 1935 seed yielded *H. sativum* as compared with 1.9% in the 1934 seed (Table II).

Further tests were made with these two samples of Pentad wheat. After surface-sterilization, seed of each sample was sown in pots of autoclaved soil, and grown under controlled conditions in the greenhouse. The plants were lifted and examined at the end of 10 days. The results of this test, given in Table III, show that the 1934 seed germinated 100% and produced but a small percentage of diseased seedlings, while of the 1935 seed 78.0% of the kernels germinated and 89.7% of the seedlings were diseased. Thus, the results of the germination and greenhouse tests confirmed the results obtained by the modified plating-out method, and demonstrated that, although the 1934 and 1935 samples of Pentad wheat were apparently equally affected by kernel smudge, only the 1934 seed was suitable for seed purposes. These results were substantiated by field tests with 1934 and 1935 seed of Pentad wheat at Winnipeg in 1936 (Table X).

TABLE III

The effect of kernel smudge on germination, and on the incidence of disease in Pentad wheat grown in sterile soil under greenhouse conditions

Crop year	Dominant fungus in seed	Percentage of seeds germinated	Percentage of plants diseased	Root-rot disease rating
1934	*Alternaria* spp.	100.0	15.0	6.2
1935	*H. sativum*	78.0	89.7	52.4

Pathogenicity of Fungi Isolated from Discolored Seed

Several workers have attempted to establish the pathogenicity of fungi isolated from discolored kernels of wheat and other cereals. In this earlier work various methods of inoculating plants with these fungi were employed. Louise Stakman (45) inserted the spores of a *Helminthosporium*, presumably *H. sativum*, between the glumes of Marquis wheat, and covered the infected

parts of the head with moist cotton and oiled paper caps for 48 hr. The mature kernels of heads thus inoculated were badly discolored. Evans (15) applied a water suspension of *H. sativum* spores to the heads of Acme and another wheat (unnamed), and enclosed the inoculated heads in glassine bags for one or two days. Many discolored kernels developed. Christensen (8) obtained many discolored kernels in cereal and grass varieties when he sprayed the growing plants with a water suspension of *H. sativum* spores. He found that the reactions of these hosts to other manifestations (leaf blotch and root rot) of the disease caused by this fungus were not correlated with the amount of disease developed in the seed. Henry (20) obtained diseased kernels when he placed dry spores or mycelium of *H. sativum* on the heads of wheat. He sprayed the heads with sterile water before and after inoculation. Scott and Sallans (40) found that wheat kernels would become discolored if a water suspension of *H. sativum* was sprayed on the heads, or injected between the glumes of the florets. Sallans (39), using similar techniques, induced infection of wheat kernels by a species of *Alternaria*. Mead (27), by employing any one of the following methods, namely, spraying the heads with, or immersing them in, a water suspension of spores of *H. sativum*, or placing, by means of a pipette, drops of the spore suspension between the spikelets and rachides, was able to induce kernel infection in barley with each of the following fungi: *H. sativum*, *Gibberella Saubinetii*, *Fusarium* sp., and *Alternaria* sp. Peterson* produced kernel smudge by injecting a spore suspension of either *H. sativum* or *Alternaria tenuis* into the florets of wheat. Each fungus was subsequently recovered from the discolored kernels of the inoculated plants.

In the present studies, kernel smudge has been produced in five varieties of wheat by the employment of any one of the following methods: (i) dusting spores of *H. sativum* on the glumes of growing plants, (ii) spraying the wheat spikes with a water suspension of spores of this fungus, (iii) injecting, by means of a hypodermic needle, the spore suspension into the leaf sheath enclosing the spike of plants in the "boot" stage of growth. Plants inoculated by either of the first two methods were incubated, immediately after inoculation, in moist chambers for two days; and then placed on a greenhouse bench and grown to maturity. The inoculated plants in all cases produced a large number of discolored kernels, from which *H. sativum* was recovered.

Pathological Histology

Bolley (3) was one of the first to observe fungus hyphae within the integuments, and around the embryo, of discolored kernels of wheat. Later (5), in referring to *Alternaria* sp. particularly, he showed that this fungus first penetrated the integuments, spreading afterwards in the spaces surrounding the embryo. He noticed, furthermore, that the scutellum often became diseased, in which case the food supply contained in the endosperm of the kernel was made unavailable to the germinating embryo. Owing to the lack

* *Unpublished data of Dr. R. F. Peterson, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.*

of food supply the young seedling was frequently unable to break through the integuments, and, when it did, it was weak and easily destroyed by pathogenic fungi such as are common in the soil. Weniger (51) found that the mycelium of *Helminthosporium sativum* was similarly distributed in the discolored kernels of *durum* wheat, and that during germination the plumule and rootlets became diseased prior to their emergence from the seed.

Peyronel (34) showed that the first symptom of disease in a kernel was a discoloration of the lodicules, organs in close proximity to the scutellum. He found at the base of these lodicules a tissue of thin-walled cells, rich in nutrients. After fertilization of the flower occurred, this tissue withered and became a very suitable substratum for the growth of fungi, the mycelium of which often penetrated the dead cells and then invaded the integuments over the embryo. Peyronel believed that the rapidity of desiccation of the lodicular tissues was a determining factor in the development of seed discolorations, a factor which he thought would affect the amount of disease in different seasons. He found also that the discoloration of the integuments was due to the formation of a brown pigment, and, although the pigment was confined chiefly to the layer of horizontal cells overlying the epicarp, it sometimes occurred in the epicarp tissue as well. The cells of the aleurone layer were also often discolored, but not those of the embryo. Dastur (12) found that, in wheat kernels infected with *H. sativum*, the fungus hyphae invaded the kernel integuments but did not reach the embryo. This freedom of the embryo from infection, he concluded, explained why many discolored kernels germinated normally.

Christensen (8) found that the mycelium of *H. sativum* within the seed of wheat remained viable for at least six and one-half years. In the present study it has been demonstrated that the mycelium of *H. sativum* and *Alternaria* spp. grew well after a dormancy of four years within the kernels. The evidence indicates that these fungi can maintain themselves in a dormant condition for several years within infected seed of cereals.

Factors Influencing the Development of Kernel Smudge

The prevalence of kernel smudge in a cereal crop is probably determined by the combined action of a number of factors. In the present investigations three of these factors, namely, air-borne inoculum, stage of host maturity, and varietal resistance, were given especial consideration.

Air-borne Inoculum

Stakman *et al.* (47), in their studies on the spore content of the upper air, observed that spores of many fungi, including *Alternaria* and *Helminthosporium*, were often lifted to considerable heights and thus could be dispersed by air currents over wide areas. Little definite information is available in the literature concerning the seasonal variation of air-borne inoculum of these fungi and the relation of such inoculum to the prevalence and development of kernel smudge. During the course of the present investigation, an attempt

was made to gather information concerning the relative number of spores of *Alternaria* and *H. sativum* in the air over Manitoba.

Each year, from 1932 to 1936, during the summer months, vaselined microscope slides were exposed daily in spore traps located at three widely separated stations in Manitoba (Winnipeg, Morden, and Brandon). The spores of *Alternaria* and *H. sativum* intercepted by one square inch of slide surface during each exposure period of 24 hr. were counted. These counts were used to compute the averages for intervals of one week each during the growing season. There is much of interest and value in these spore-counting data, but a detailed discussion of the results would occupy too much space, therefore, only the results of the spore counts made at Winnipeg during these years are presented (Table IV, Fig. 1).

The data in Table IV show that during June, July, and August, of each year, spores of *Alternaria* were numerous in the atmosphere. An appreciable increase in the number of these spores occurred during late July and early

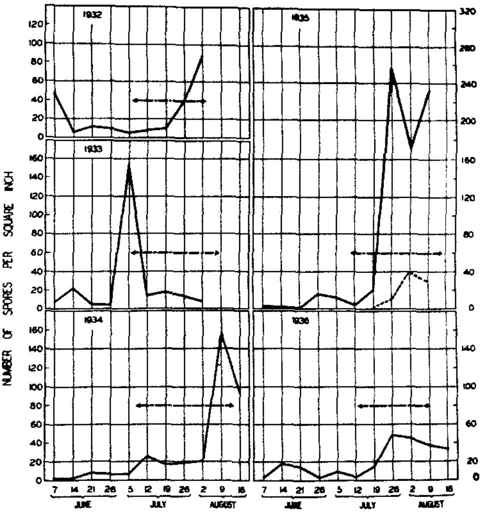

FIG. 1. *The seasonal variation in number of air-borne spores of kernel-smudge fungi, as represented by weekly averages of daily spore counts in June, July, and August, of each of the 5 years, 1932-1936. Alternaria spp. represented by a continuous line, Helminthosporium sativum (1935) by a broken line. The interval between date of heading and harvest each year is indicated by a broken, arrowhead line.*

August in 1932, 1934, 1935, and 1936. Sallans (39) made counts of air-borne spores of *Alternaria* during June, July, and August in 1928 and 1929, at Indian Head and Saskatoon, Saskatchewan, and found a similar sharp increase in the number of spores during late July. This increase was particularly noticeable in 1928 at Indian Head.

While spores of *Alternaria* were intercepted in large numbers on the slides during each year of this investigation, spores of *H. sativum* were usually intercepted only in very small numbers. In Fig. 1, therefore, graphs showing the occurrence of this fungus on the slides in 1932, 1933, 1934, and 1936, are omitted. In 1935, however, there was a very decided increase in the number of *H. sativum* spores intercepted by the slides at three stations, and, therefore a graph showing the occurrence of this fungus in 1935 is given.

TABLE IV

THE SEASONAL VARIATION IN THE NUMBER OF SPORES OF KERNEL SMUDGE FUNGI CAUGHT ON GLASS SLIDES AT WINNIPEG, MAN., DURING THE FIVE YEARS 1932–1936. (WEEKLY AVERAGES OF DAILY COUNTS OF NUMBER OF SPORES ON ONE SQUARE INCH OF SLIDE DURING JUNE, JULY, AND AUGUST)

Week ending	1932		1933		1934		1935		1936	
	Alternaria	*H. sativum*	*Alternaria*	*H. sativum*	*Alternaria*	*H. sativum*	*Alternaria*	*H. sativum*	*Alternaria*	*H. sativum*
June 7	48.2	0.0	7.0	0.0	2.0	0.0	2.9	0.2	2.1	0.1
14	5.1	0.0	22.0	0.3	2.0	0.0	1.4	0.0	18.1	2.4
21	11.4	0.0	4.0	0.6	8.0	0.0	0.7	0.0	14.3	0.1
28	9.6	0.0	3.1	0.0	7.0	0.0	15.6	1.0	2.4	0.3
July 5	3.7	0.0	156.0	0.0	7.0	0.0	12.9	0.9	9.0	0.7
12	8.3	0.0	15.5	0.0	26.3	0.0	3.0	0.0	2.4	0.3
19	12.9	0.0	18.1	0.0	18.4	0.0	19.9	1.8	15.0	0.4
26	40.3	0.0	13.2	0.0	19.1	0.0	257.9	11.2	47.1	0.8
Aug. 2	88.3	1.7	8.2	0.0	19.5	0.0	170.9	40.8	45.7	11.5
9	—	—	—	—	149.3	0.0	229.7	35.7	38.5	2.0
16	—	—	—	—	93.2	0.0	—	—	35.2	2.0

An examination of the data on daily spore counts of *Alternaria* and *H. sativum*, presented graphically in Fig. 1, shows that, in each year (except at Winnipeg in 1933), the largest numbers of spores of these fungi were present in the air over Manitoba during the period of wheat growth from heading to maturity. This period of growth for Marquis wheat is indicated in each graph in Fig. 1 by a broken, arrow-head line. At Winnipeg, in 1933, a very marked increase in the number of *Alternaria* spores occurred during the last week of June and the first week of July, before the plants had reached the heading stage. The cause of this exceptionally early increase is not clearly understood.

From daily counts of *Alternaria* spores, it was evident that the atmospheric spore load varied greatly from day to day. An examination of the meteorological data for the summer months showed that there was a close correlation

between wind direction and the number of *Alternaria* spores on the slides. The greatest numbers were caught after a southerly wind had blown for one or two days. A change of wind direction invariably resulted in an appreciable decrease in the number of spores. It was concluded, therefore, that the greater part of the spores of *Alternaria* originated in areas to the south of Manitoba, and that these air-borne spores were widely disseminated by winds over the grain-growing areas of this province.

Although the spore counts are an index of the relative number of spores in the air, they are not necessarily a reliable index of the number of spores that are deposited on living hosts in any given area. The spore traps were not designed to measure the number of spores deposited at ground level, or even to indicate the number of spores in the atmosphere at the level of grain crops. Thus, the data presented in Table IV are only of value in so far as they give a general indication of the prevalence of fungus spores in the air from day to day. Nevertheless, the value of such information in predicting destructive outbreaks of plant diseases has been demonstrated. The occurrence of a severe outbreak of kernel smudge (*H. sativum* type) in Manitoba was predicted in 1935 from the information gained in a study of the spore content of the air. As indicated earlier, this type of kernel smudge was epidemic in that year.

The number of *Alternaria* spores present in the air during the summer of 1935 greatly exceeded the number of *H. sativum* spores and consequently it was expected that a large proportion of the discolored kernels would be infected by *Alternaria*. Isolation studies (Table II) did not confirm this expectation, because the majority of the diseased kernels yielded *H. sativum*.

Why the *H. sativum* type of kernel smudge predominated in 1935 is not altogether clear. From the work of Bolley, Dastur, Peyronel, and Weniger, it appears that infection of wheat kernels by *H. sativum* and *Alternaria* proceeds in a somewhat similar manner. If air-borne inoculum of both fungi is present, as in 1935, it would be expected that many of the kernels would probably be infected by both fungi. On this assumption both fungi should be isolated from such kernels, for the technique of isolation used by the writers does not favor the isolation of one more than the other. It would appear, therefore, that some factor, probably an antagonism between the two fungi in the seed, may have been responsible for the high percentage of *H. sativum* isolates from the 1935 seed.

There is some evidence in support of this hypothesis. It has been the experience of the present writers that, when *Alternaria* spp. and *H. sativum* are cultured together, the *Alternaria* cultures are not compatible with those of *H. sativum*, that is, the association hinders the growth of *Alternaria*. Recently Johnson* found that when colonies of *Alternaria* sp. and *H. sativum* were cultured together in the same Petri dish the adverse influence of *H. sativum* on *Alternaria* increased with a decrease in the distance separating

* *Unpublished data of Dr. T. Johnson, Dominion Rust Research Laboratory, Winnipeg, Manitoba.*

the two inocula on the agar. When the inocula were placed side by side the growth of *Alternaria* was very limited while that of *H. sativum* was not hindered in the least.

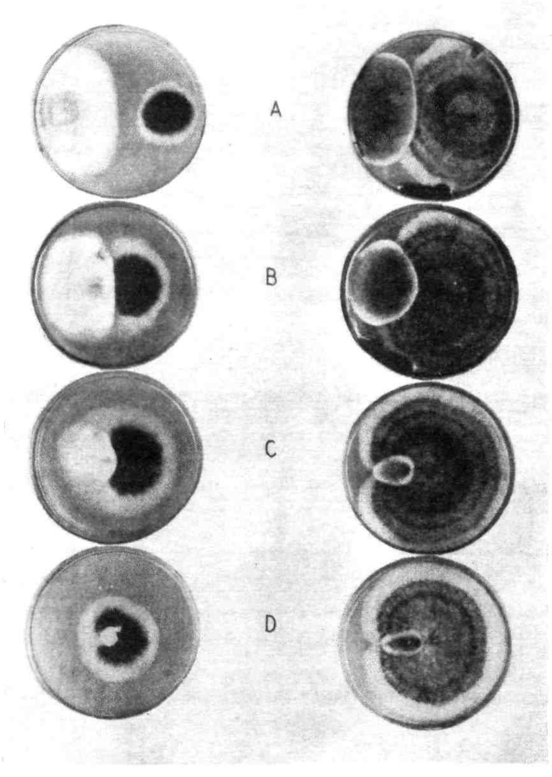

FIG. 2. *The inhibiting action of Helminthosporium sativum on Alternaria tenuis. Growth of colonies on Czapek's agar (left) and potato-dextrose agar (right) after 8 days. A. Inocula placed 6 cm. apart. B. Inocula placed 2.2 cm. apart. C. Inocula placed 1 cm. apart. D. Inocula placed side by side on surface of agar.*

The experiments of Johnson have been repeated, and his results confirmed, by the present workers. Two fungi, *Alternaria tenuis* and *H. sativum*, isolated from wheat kernels affected by kernel smudge, were cultured on potato-dextrose agar and Czapek's agar. The inoculums of the two fungi were placed at four different distances apart. Cultures grown for a period of one week at room temperature appeared as shown in Fig. 2. Repetition of the test produced similar results.

On the basis of the results obtained by Johnson and the writers, it appears possible that, in the absence of *H. sativum*, infection of the kernels by *Alternaria* occurs in the normal way, but when infection by both fungi is more or less simultaneous the repressive action of *H. sativum* on *Alternaria* prevents the complete establishment of the latter fungus in the kernel, while the other grows more or less normally. It is possible, too, that a kernel already infected by *Alternaria* may be invaded, partly at least, by *H. sativum*. Furthermore, it may be assumed that, when attempts are made to isolate these fungi from diseased kernels, the outward growth of *Alternaria* is inhibited, or at least retarded by the antagonistic action of *H. sativum*. On this assumption, it could be expected that the number of isolates of *H. sativum* from the 1935 crop of wheat, previously referred to, would greatly exceed those of *Alternaria*, even though the latter fungus had the greater chance to infect the kernels. The results of 1935 (Table II) substantiate this expectation, for there was only slight evidence of *Alternaria* infection in the 1935 seed. Although the above hypothesis is based on analogy, it seems to be a reasonable explanation of the problem encountered in 1935. The fact that *H. sativum* inhibited *Alternaria tenuis* in a similar manner, whether potato-dextrose agar or Czapek's agar was used, adds further weight to the conviction that the hypothesis is at least partially correct, as the chances that the same phenomenon would occur in the kernels of cereals are greatly increased.

Stage of Host Maturity

Kernel smudge is essentially a disease of the seed. Apparently, in nature, the interval during which infection of the seed may take place is restricted to the period of kernel development. Christensen (9) stated that *H. sativum* can infect the wheat kernel during any stage of its growth. Peyronel (34) found that the fungi causing kernel discoloration infected the growing kernels within a few days after the flowers had been fertilized. Scott and Sallans (40) showed that most of the infection occurred during the blossoming period of the flowers, but also that infection could occur at any later stage of kernel growth. Sallans (39) found that the characteristic discoloration appeared most often when the kernels were inoculated during or after the "soft dough" stage of growth. Rosella (38) observed that *H. sativum* invaded barley spikes while they were still enclosed by the leaf sheaths. The fungus penetrated the sheaths, reached the young inflorescence, passed through the glumes and rachides, and finally invaded the kernels. Henry (20) showed in his experiments that *H. sativum* invaded wheat kernels chiefly during the "milk" stage of their growth.

The actual time during which the kernels become infested under field conditions is still a matter of conjecture. In the case of the *H. sativum* type of kernel smudge, the kernels are invaded either indirectly, as the work of Rosella (38) and the present studies have shown, or directly, as a number of workers (8, 14, 27, 40, 45) have demonstrated. The species of *Alternaria* causing kernel smudge do not appear to be so actively parasitic as *H. sativum*, for they do not ordinarily attack the glumes and rachides until these have withered and died. Therefore, it is reasonable to assume that under ordinary conditions the infection of the kernel by *Alternaria* spp. is more or less direct, that is, the fungus initially comes in contact with some part of the growing kernel. The kernel may become infected either when the florets are expanded during the blossoming period, or when, at or near plant maturity, the increase in kernel size separates the glumes.

There are two reasons for assuming that, under Manitoba conditions, infection of the kernel takes place during the more mature stages of plant growth. First, there is very little available inoculum at the time of the first opening of the florets (Fig. 1 and Table IV), and second, it has been found that the infected kernels are usually plump and well developed (Table V). From the literature on kernel discolorations in cereals, however, it appears that infection of the kernel by *H. sativum* leads to reduction in size of the kernel. On the other hand, the writers, working under greenhouse conditions, found that it was only when they inoculated very immature kernels with *H. sativum* that the mature kernels were shrunken. During the years of the present investigation, shrunken kernels with discolorations typical of kernel smudge were seldom found, but the disease was commonly observed on large kernels.

Further evidence, that under Manitoba conditions kernel smudge occurs from late infection of the kernels, can be obtained by examining the relation between stem rust of wheat and the prevalence of kernel smudge. It is a well known fact that severe attacks by stem rust result in an appreciable reduction in kernel size. In the present investigation the shrunken grain from a rusted crop was examined to determine the prevalence of discolored kernels. Very few shrunken kernels were found to be discolored. It seems that, owing to the reduced size of the kernels, the enveloping glumes did not separate widely as the kernels matured, and hence an avenue for the kernel smudge fungi was not provided. The negative correlation found by Goulden (17) between the amount of kernel smudge in a wheat variety and its susceptibility to stem rust may be explained on this basis.

Varietal Resistance

Bolley (5) showed that, in the development of seed discolorations, the variety of host is an important factor: the hard wheats were generally more susceptible than the soft wheats. Dastur (12) found that wheat varieties differed in their susceptibility to the *H. sativum* type of this disease. Christensen (8) stated that the reactions of different wheat varieties to *H. sativum*,

as defined by the occurrence of leaf lesions, were not correlated with the percentages of discolored kernels in the grain. Other workers (15, 52) have shown that the *durum* wheats are more susceptible to seed discoloration than are wheats of the *vulgare* type, a finding which has been confirmed by the present studies (Table I).

In 1931, Peterson* found a wide range of susceptibility to kernel smudge among wheat varieties. In general, varieties of the durum type were more susceptible to the disease than were varieties of the *vulgare* type. In a series of pure lines of a *durum* × *vulgare* (Pentad × Marquis) wheat cross, the susceptibility of the *durum* parent was carried over to most of these lines, although a few of them showed a high resistance to the disease. In other wheat crosses (H-44-24 × Reward, and Double Cross × Ceres), tested in 1931, a similar result was obtained. The experiments were repeated in 1932 with results very similar to those of 1931. In 1933, he made greenhouse tests to determine the reaction of wheat varieties to kernel smudge. These tests showed that the greenhouse reactions of certain wheat varieties to *H. sativum* and *Alternaria* sp. were closely correlated with the field reaction of these varieties.

Goulden (17) made several interesting observations on the relation of host variety to the amount of kernel smudge. His results, based on an analysis of the relation between several pairs of variables in 52 pure lines of a H-44-24 × Marquis cross, demonstrated that there was a high degree of correlation between certain pairs of these variables. These results established that the percentage of kernel smudge in the crop was significantly, but negatively, correlated with (i) infection by Form 21 of wheat stem rust (*Puccinia graminis tritici* Erikss. and Henn.), (ii) seed color, and (iii) length of time required to mature the crop. There was a slight, but significant, positive correlation between yield and the percentage of kernel smudge.

Effect of Kernel Smudge on Size and Weight of Kernels

Peyronel (34), Rosella (37), and Waldron (49) showed that discolored wheat kernels are frequently larger and heavier than are sound kernels. Tests were made to determine whether a similar situation prevailed under Manitoba conditions. The seed of Pentad wheat, produced in sulphur-dusted and undusted plots at Winnipeg during 1933, 1934, and 1935, was used in these tests. From the threshed grain of both dusted and control plots of each year, three samples were taken at random. For each of these samples the weight per 1000 kernels of each type of seed (healthy and smudged) was determined. The average 1000-kernel weight for each group of three samples in these tests is given in Table V.

The data in Table V confirm the findings of previous workers (34, 37, 49), and show that, in Manitoba, kernel smudge is usually very prevalent in fully developed kernels of wheat. In each year of the test the discolored kernels were heavier than the healthy kernels, although the difference in weight

* *See reference, page 91.*

TABLE V

Relative weight of 1000 kernels of healthy and discolored seed from sulphur-dusted and undusted plants of Pentad wheat 1933, 1934, and 1935. (Average weight in grams of three 1000-kernel samples)

Crop year	Plants dusted with sulphur			Plants not dusted		
	Healthy seed	Diseased seed	Increase in weight of diseased seed	Healthy seed	Diseased seed	Increase in weight of diseased seed
1933	26.9	27.8	0.9	27.0	28.0	1.0
1934	29.7	33.9	4.2	29.1	32.6	3.5
1935	16.5	20.2	3.7	15.3	18.5	3.2
Mean	24.4	27.3	2.9	23.8	26.4	2.6

varied from year to year. Dusting growing plants with sulphur resulted in a slight increase in kernel weight, but it did not disturb materially the relation between the weight of healthy and diseased kernels.

Influence of Kernel Smudge on Plant Growth, Incidence of Seedling Blight and Root Rot, and on Yield

Experimental Methods

Field experiments to determine the effect on the subsequent crop of planting seed affected by kernel smudge were made in 1932, 1933, and 1934. The experiments were designed to study several aspects of the disease. The system of plot replication used consisted of six randomized blocks of at least eight plots each. Within each block there was complete randomization of varieties and kinds of seeds (diseased and healthy). The individual plots consisted of two rod rows placed one foot apart. In one of these rows a predetermined quantity (15 gm. of wheat of the *vulgare* type and 18 gm. of the *durum* type) was sown, while one hundred kernels of the same variety and same kind of seed were sown, spaced about two and one-half inches apart, in the other row. The former row furnished data relative to yield and the amount of kernel smudge in the threshed grain of the plot, while the latter provided information concerning the number of plants and the degree of infection on individual plants.

Notes on the number of plants and amount of root rot were obtained from ten to fifteen days before harvest. The plants in the 100-seed row of each plot were pulled, counted, and examined individually, and the severity of root-rot infection recorded. The degree of root rot in each plot was subsequently determined by the formula (modified) of McKinney (25), and the experimental data analyzed by the procedure described by Fisher (16). To estimate the odds of significance, however, Snedecor's (44) F value, the direct ratio of the variances, was used. The complete experiment was made at Winnipeg in 1932, and repeated in 1933 and 1934.

THE EFFECT OF KERNEL SMUDGE ON SEED GERMINATION AND PLANT EMERGENCE

Puchner (35) found that discolored kernels of barley germinated poorly, and, after germination, often produced seedlings with disease lesions on the leaves. Bolley (3, 4, 5) stated that wheat seedlings arising from diseased kernels were usually weak and, when grown under field conditions, subject to disease. Güssow (18) found that discolored wheat seed germinated as well as did healthy seed, but that the seedlings resulting from the discolored seed were not very thrifty. Louise Stakman (45, 46), Christensen (8), Christensen and Stakman (10), Weniger (51), Drechsler (14), Henry (20), Simmonds (41), and Scott and Sallans (40), found that wheat kernels attacked by *H. sativum* germinated poorly, and that, in most cases, diseased seedlings resulted from such seed. Dastur (12) showed that the value of a crop was appreciably reduced when seed infected with *H. sativum* was planted. He observed that the discolored seed germinated poorly, and that the seedlings from such seed usually became blighted. He found, also, that the amount of injury to the seedlings depended to a considerable extent on the time of seeding and on the weather conditions prevailing at the time of seed germination. Rosella (37) found that discolored wheat and barley kernels germinated better in sand than on nutrient agar in Petri dishes. Miège (29) obtained no differences in germination when healthy and discolored wheat seed was planted in field soil. The results of a three-year test made by Stening (48) showed that discolored wheat seed germinated about 5.5% less than did healthy seed. Waldron (50) obtained only slight reductions in germination when discolored wheat seed was planted. Machacek (23) found that discolored kernels selected from a number of wheat varieties germinated poorly, and observed that such seed, when surface-sterilized and plated-out on nutrient agar or planted in sterile soil, gave rise to seedlings the coleoptiles and rootlets of which were diseased.

One of the objects of the field experiments made at Winnipeg in 1932, 1933, and 1934, was to determine the effect of sowing discolored seed on plant emergence in wheat. Several varieties of wheat were planted. The results for the three years are given in Table VI. They show that, although the differences in plant emergence observed between healthy and diseased seed were not significant in any given year of the test, there was a general tendency for the discolored seed to give lower emergence than the healthy seed. However, as the discolored seed used in 1932, 1933, and 1934, was mostly infected with *Alternaria* sp., it was not to be expected that there would be any marked difference in the germinability of healthy and discolored seed planted in these years. If the seed had been infected by *H. sativum*, however, it is probable that a significant difference in germination of the healthy and diseased seed would have been obtained, as the results in Table III lead one to expect, and those in Table X substantiate.

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TABLE VI

EFFECT OF PLANTING HEALTHY AND DISEASED (KERNEL SMUDGE) SEED OF WHEAT IN FIELD PLOTS ON PLANT EMERGENCE IN 1932, 1933, AND 1934

Analyses of Variance

Year	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
1932	Replicates	5	1415.35	283.07		
	Varieties	3	179.73	59.91	—	—
	Error (a)	15	945.90	63.06		
	Total	23	2540.98			
	Seed (healthy and diseased)	1	143.52	143.52	3.55	4.35
	Seed × varieties	3	49.06	16.35	—	—
	Error (b)	20	808.92	40.44		
	Total	24	1001.50			
1933	Controlled error	8	1485.24	185.65		
	Varieties	5	2880.24	576.04	9.91	2.66
	Error (a)	22	1278.68	58.12		
	Total	35	5644.16			
	Seed (healthy and diseased)	1	153.12	153.12	2.57	4.17
	Seed × varieties	5	1069.46	213.89	3.58	2.53
	Error (b)	30	1790.92	59.69		
	Total	36	3013.50			
1934	Controlled error	8	1660.91	207.61		
	Varieties	3	368.23	106.08	1.20	3.49
	Error (a)	12	1064.34	88.69		
	Total	23	3043.48			
	Seed (healthy and diseased)	1	11.02	11.02	—	—
	Seed × varieties	3	295.23	98.41	2.15	3.10
	Error (b)	20	914.25	45.71		
	Total	24	1220.50			

Summary of Results (Percentage of Plants Emerged)

Variety	1932		1933		1934	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Mindum	—	—	—	—	68.3	73.5
Marquis	—	—	67.3	50.2	—	—
Ceres	—	—	66.3	69.3	—	—
Reward	—	—	56.7	56.3	—	—
Pentad	74.8	71.8	58.8	64.7	70.0	63.8
Acme	69.2	66.7	47.0	46.5	68.5	63.0
Pentad × Marquis (R.L. 726)	74.0	67.2	63.2	54.8	70.0	76.2
Pentad × Marquis (R.L. 723)	72.0	70.1	—	—	—	—
Mean*	72.5	69.0	59.9	57.0	69.2	69.1
Standard error of varieties	—	—	3.11	3.11	—	—

* *The difference between means of healthy and diseased seed not significant in 1932, 1933, and 1934*

The Effect of Kernel Smudge on the Development of Seedling Blight and Root Rot

As pointed out in the preceding section of this paper, most investigations with discolored seed of cereals have shown that, in general, such seed gives rise to diseased seedlings. The results of some, however, are not in accord with this finding. In the present studies it has been demonstrated (Table VI) that, under field conditions in Manitoba during 1932, 1933, and 1934, the differences in plant emergence between healthy and discolored seed were not statistically significant. The results of preliminary tests (Table II) indicate that the conflicting results obtained by earlier workers may easily be accounted for by the supposition that the seed used by some was infected with *Alternaria* while that used by others was infected with *H. sativum*. It has been clearly demonstrated (Table III) that the amount of seedling disease resulting from kernel smudge depends upon the fungus with which the seed is infected.

Further information concerning the effect of planting discolored seed of wheat on the incidence of seedling blight and root rot was obtained from the field experiments of 1932, 1933, and 1934. The summarized results of these experiments are given in Table VII.

The differences observed in the amount of root rot in these years between plots planted with discolored seed and adjoining plots planted with healthy seed were not statistically significant. There was, however, slightly more disease in the plots planted with diseased seed. The discolored seed used in these experiments was infected with *Alternaria* spp. From the results presented in Table VII, it may be concluded that seed attacked by kernel smudge of the *Alternaria* type does not produce root rot in the subsequent crop.

The Effect of Kernel Smudge on Yield

It has been demonstrated by a number of workers (3, 8, 10, 14, 20, 35, 40, 41, 45, 51) that wheat seed infested by *H. sativum* produced fewer plants than healthy seed. These plants were usually more affected by root rot than were plants from healthy seed. On the other hand, Dastur (12) showed that infection of wheat seed by this fungus was not always a determining factor influencing crop yield. He pointed out that the amount of root rot developed on the plants was determined to a great extent by the weather conditions which prevailed during the early stages of plant growth. Stening (48), Miège (29), and Waldron (50) showed that the planting of discolored seed did not influence the yield of wheat.

In the present investigations an effort was made to determine whether, under field conditions, the planting of seed affected by the *Alternaria* type of kernel smudge affected the yield. The yield data, given in Table VIII, were taken from the field experiments made at Winnipeg in 1932, 1933, and 1934. They show that in 1932 the yield of plants from healthy seed was significantly higher (4.0 bushels per acre) than the yield of plants from diseased seed. In 1933 and 1934, the differences in yield observed between healthy

TABLE VII

EFFECT OF PLANTING HEALTHY AND DISEASED (KERNEL SMUDGE) SEED OF WHEAT IN FIELD PLOTS IN 1932, 1933 AND 1934 ON THE INCIDENCE OF ROOT ROT

Analyses of Variance

Year	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
1932	Replicates	5	901.85	108.37		
	Varieties	3	159.23	53.07	1.07	3.29
	Error (a)	15	740.90	49.39		
	Total	23	1801.98			
	Seed (healthy and diseased)	1	63.02	63.02	2.67	4.35
	Seed × varieties	3	98.73	32.91	—	—
	Error (b)	20	470.75	23.53		
	Total	24	632.50			
1933	Controlled error	8	1004.15	125.52		
	Varieties	5	1443.71	288.74	9.46	2.66
	Error (a)	22	671.08	30.50		
	Total	35	3118.94			
	Seed (healthy and diseased)	1	85.80	85.80	2.73	4.17
	Seed × varieties	5	573.07	114.61	3.64	2.53
	Error (b)	30	943.99	31.46		
	Total	36	1602.86			
1934	Controlled error	8	1202.85	150.30		
	Varieties	3	805.94	268.64	3.98	3.49
	Error (b)	12	810.05	67.50		
	Total	23	2818.84			
	Seed (healthy and diseased)	1	14.08	14.08	—	—
	Seed × varieties	3	238.72	79.57	2.54	3.10
	Error (b)	20	625.64	31.28		
	Total	24	878.44			

Summary of Results (Disease Rating)

Variety	1932		1933		1934	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Mindum	—	—	—	—	27.2	22.0
Marquis	—	—	55.5	68.6	—	—
Ceres	—	—	56.8	54.9	—	—
Reward	—	—	64.2	64.8	—	—
Pentad	51.6	51.1	63.9	59.3	28.8	33.2
Acme	54.0	57.6	70.3	71.8	27.2	31.2
Pentad × Marquis (R.L. 726)	49.8	56.1	60.8	65.3	20.2	19.6
Pentad × Marquis (R.L. 723)	51.5	51.1	—	—	—	—
Mean*	51.6	53.6	61.9	64.1	25.3	26.5
Standard error of varieties	—	—	2.25	2.25	3.55	3.35

* *The difference between means of healthy and diseased seed not significant in 1932, 1933, and 1934.*

TABLE VIII

EFFECT OF PLANTING HEALTHY AND DISEASED (KERNEL SMUDGE) SEED OF WHEAT IN FIELD PLOTS IN 1932, 1933 AND 1934 ON YIELD

Analyses of Variance

Year	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
1932	Replicates	5	913.38	182.67		
	Varieties	3	667.40	222.46	10.20	3.29
	Error (a)	15	326.95	21.79		
	Total	23	1907.73	426.92		
	Seed (healthy and diseased)	1	137.70	137.70	10.00	4.35
	Seed × varieties	3	102.68	34.22	2.48	3.10
	Error (b)	20	275.57	13.77		
	Total	24	515.95			
1933	Controlled error	8	327.27	40.90		
	Varieties	5	619.84	123.96	13.64	2.66
	Error (a)	22	199.98	9.09		
	Total	35	1147.09			
	Seed (healthy and diseased)	1	2.45	2.45	1.75	4.17
	Seed × varieties	5	4.43	0.89	—	—
	Error (b)	30	42.01	1.40		
	Total	36	48.89			
1934	Controlled error	8	3123.94	390.49		
	Varieties	3	3178.41	1059.47	202.19	3.49
	Error (a)	12	62.88	5.24		
	Total	23	6365.23			
	Seed (healthy and diseased)	1	44.66	44.66	—	—
	Seed × varieties	3	9.88	3.29	—	—
	Error (b)	20	1262.19	63.11		
	Total	24	1316.73			

Summary of Results (Yield in Bushels per Acre)

Variety	1932		1933		1934	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Mindum	—	—	—	—	22.2	26.3
Marquis	—	—	14.4	13.5	—	—
Ceres	—	—	17.0	16.9	—	—
Reward	—	—	8.0	7.0	—	—
Pentad	52.1	45.3	12.7	13.1	41.1	42.2
Acme	51.6	45.8	13.1	13.0	44.4	46.1
Pentad × Marquis (R.L. 726)	41.5	41.4	10.8	10.3	29.7	32.8
Pentad × Marquis (R.L. 723)	46.7	43.3	—	—	—	
Mean	47.9	43.9	12.6	12.3	34.3	36.8
Standard error of mean*	0.76		—		—	
Standard error of varieties	1.90	1.90	1.23	1.23	0.93	0.93

* *The difference between means of healthy and diseased seed not significant in 1933 and 1934.*

and diseased seed were not significant. From these results it may be concluded that the planting of discolored seed, in which the disease is due to species of *Alternaria*, does not, in general, materially influence the yield.

The Effect of Planting Discolored Seed on the Occurrence of Kernel Smudge in the Subsequent Crop

Little definite information concerning the influence of planting discolored seed on the occurrence of kernel smudge in the subsequent crop is available. Stening (48) stated that discolored seed of wheat could be safely planted. There appeared to be little probability that sowing diseased seed had any direct influence on the amount of kernel smudge in the harvested grain. Dastur (12) showed that the amount of discoloration in the grain is influenced more by atmospheric temperature and moisture than by any other factor, including the planting of diseased seed.

To secure more definite information concerning the effect of planting discolored seed on the amount of disease occurring in the subsequent crop, an examination was made of the threshed grain of the field experiments of 1932, 1933, and 1934. In each of these years, the seed planted in the field tests was obtained from the crops of the preceding year. After the plants in the various plots had been harvested and the grain threshed, the percentage of discolored kernels present in each plot sample, based on a count of a 1000 kernels, was computed. The results of the three-year test, showing the percentage of discolored kernels in the grain of plants arising from healthy and discolored seed, are given in Table IX.

In 1932 there was no difference in the amount of kernel smudge in the crop resulting from healthy and diseased seed, but in 1933 and 1934 slightly more diseased kernels were found in the grain from discolored than from healthy seed. Only in 1934, however, was this difference statistically significant. It may be concluded from these results that planting seed infected with kernel smudge of the *Alternaria* type does not result in a material increase in the amount of disease in the grain of the next crop. To this extent, therefore, these results are in agreement with those of Stening (48).

The Relative Effect of the *Alternaria* and *Helminthosporium sativum* Types of Kernel Smudge in Pentad Wheat on Plant Emergence, Occurrence of Root Rot, and Yield

Discolored kernels in a number of wheat varieties grown in Manitoba during the years 1929 to 1934 yielded more or less consistently cultures of *Alternaria* species. Moreover, field tests (Tables VI–IX) showed that kernel smudge of this type caused little or no harm to the subsequent crop. These results led to the conclusion that *Alternaria* was the main cause of kernel smudge in wheat grown in Manitoba. In 1935, however, the results obtained at Winnipeg forced the writers to modify this conclusion. During the latter part of the summer of that year, spores of *H. sativum*, in greater numbers than in any of the three previous years, appeared in Manitoba (Table IV).

TABLE IX

EFFECT OF PLANTING HEALTHY AND DISEASED (KERNEL SMUDGE) SEED IN FIELD PLOTS ON THE AMOUNT OF KERNEL SMUDGE IN THE CROPS OF 1932, 1933 AND 1934

Analyses of Variance

Year	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
1932	Replicates	5	382.21	76.44		
	Varieties	3	17503.70	5834.56	153.53	3.29
	Error (a)	15	570.63	38.04		
	Total	23	18456.54			
	Seed (healthy and diseased)	1	0.10	0.10	—	—
	Seed × varieties	3	116.52	38.84	3.66	3.10
	Error (b)	20	211.71	10.58		
	Total	24	328.33			
1933	Controlled error	8	79.01	9.88		
	Varieties	5	19158.16	3831.63	506.10	2.66
	Error (a)	22	166.55	7.57		
	Total	35	19403.72			
	Seed (healthy and diseased)	1	9.32	9.32	—	
	Seed × varieties	5	78.56	15.71	—	—
	Error (b)	30	288.70	9.62		
	Total	36	376.58			
1934	Controlled error	5	1513.88	302.62		
	Varieties	3	49296.03	16432.01	214.01	3.29
	Error (a)	15	1151.77	76.78		
	Total	23	51960.88			
	Seed (healthy and diseased)	1	305.02	305.02	26.45	4.35
	Seed × varieties	3	124.43	41.48	3.60	3.10
	Error (b)	20	230.66	11.53		
	Total	24	660.11			

Summary of Results (Percentage of Smudged Kernels)

Variety	1932		1933		1934	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Mindum	—	—	—	—	24.2	27.9
Marquis	—	—	0.8	0.7	—	—
Ceres	—	—	0.9	1.8	—	—
Reward	—	—	0.6	0.7	—	—
Pentad	42.2	37.3	41.3	40.7	79.0	86.0
Acme	39.0	42.8	25.4	30.8	69.0	77.9
Pentad × Marquis (R.L. 726)	2.3	2.3	1.2	0.7	5.4	5.9
Pentad × Marquis (R.L. 723)	1.6	2.4	—	—	—	—
Mean	21.2	21.2	11.7	12.5	44.4	49.4
Standard error of mean*	—		—		0.69	
Standard error of varieties	2.52	2.52	1.12	1.12	3.57	3.57

* *The difference between healthy and diseased seed not significant in 1932 and 1933.*

When the field plots were harvested, a large percentage of the kernels in the threshed grain were found to be discolored. Isolations were then made from discolored seed of Pentad wheat grown in 1934 and 1935. The results (Table II) showed distinctly that in 1934, as in the previous years, the kernels were infected mostly with *Alternaria*, while in 1935 the seed was infected almost entirely with *H. sativum*.

A preliminary greenhouse test (Table III) showed that there was a marked difference in the amount of seedling blight that developed in the seedlings resulting from the two types of seed. The seedlings from the 1935 seed were severely blighted while those from the 1934 seed were not. In 1936, in order to clarify the greenhouse results by means of a field test, the seed of the 1934 and 1935 crops of Pentad wheat was tested in properly randomized and replicated field plots at Winnipeg. The results of this experiment are given in Table X.

TABLE X

THE EFFECT OF THE *Alternaria* AND *H. sativum* TYPES OF KERNEL SMUDGE ON EMERGENCE OF SEEDLINGS, SEVERITY OF ROOT ROT, AND YIELD OF PENTAD WHEAT UNDER FIELD CONDITIONS IN 1936

Crop year	Dominant fungus in seed	Seedling emergence, %	Disease rating (seedling)	Disease rating (adult plants)	Yield per acre, bu.
1934	*Alternaria* spp.	87.5	28.5	53.1	29.2
1935	*H. sativum*	24.9	80.6	85.3	20.6
Difference		62.6	52.1	32.2	8.6

Table X shows distinctly that the *H. sativum* type of kernel smudge (1935 seed) caused considerably more seedling blight and root rot in the crop than did the *Alternaria* type of this disease (1934 seed). Thus there is little reason to doubt that the disagreements in the findings of previous workers have been due to the fact that the seed which these workers had used was infected by at least two different kinds of fungi, one of which was pathogenic to the growing plants, while the other was not. The similarity in symptoms produced by *Alternaria* and *H. sativum* in the seed of cereals has, no doubt, led many of these workers to believe that they were dealing with a disease produced by a single fungus.

Control

The literature dealing with the control of kernel smudge in grain and the prevention of adverse consequences to the next crop is not very extensive. Bolley (5) advised crop rotation as a means of controlling this disease, because he found seed discolorations very prevalent in cereals grown on "wheat-sick" soil. McKinney (26) made similar recommendations. Christensen (9), Goulden (17), Miège (29), and Weniger (51) showed that wheat varieties differed in susceptibility to the disease. Peterson* showed that these differences were heritable, thereby suggesting that varieties of wheat resistant to kernel smudge may be produced.

* *See reference, page 91.*

With the exception of the work of Bolley (5) and McKinney (26), no attempts have been made to prevent the development of kernel smudge in cereal crops. A few workers, however, have attempted to control the disease by destroying the fungus within the discolored seed. Atanasoff and Johnson (1) used dry heat for this purpose. Peyronel (34) reported that the practice of treating diseased seed with copper fungicides was extensively followed in Sicily, and suggested that good results might follow the use of copper dusts for this purpose. Waldron (50) attempted to prevent seedling blight, and to increase emergence of seedlings arising from discolored seed, by treating such seed with New Improved Ceresan. This method of control did not appear satisfactory.

The Effect of Sulphur Dust Applied to the Growing Plants

At Winnipeg, an attempt was made to control kernel smudge by dusting the growing crop with sulphur dust. Grain samples from field plots of Marquis wheat, which received, during the growing seasons of 1929, 1930, and 1931, periodic dustings with finely divided sulphur to prevent stem rust, contained in the latter two years a smaller percentage of discolored kernels than did grain samples from plots that received no dust (Table XI).

TABLE XI

The effect of dusting growing plants with sulphur on the percentage of kernel smudge in Marquis wheat. (Percentages based on 1000-kernel counts)

Year	Treatment	Amount of dust, lb. per acre	Dusting interval, days	Total dustings	Percentage of kernel smudge
1929	None	0	—	0	0.3
	Dusted	45	3	9	0.5
1930	None	0	—	0	4.9
	Dusted	15	21	3	1.7
	Dusted	15	14	5	1.8
	Dusted	15	7	9	1.2
	Dusted	15	3	15	0.9
	Dusted	30	14	3	1.1
	Dusted	30	7	5	1.1
	Dusted	30	3	9	0.5
1931	None	0	—	0	0.9
	Dusted	30	3	15	0.6

The results in Table XI indicated that dusting growing plants with sulphur controls kernel smudge to some extent. However, as Marquis wheat was not very susceptible to kernel smudge under Manitoba conditions in 1929, 1930, and 1931, further tests with other varieties of wheat seemed desirable. In 1932, therefore, sulphur-dusting tests were designed in which the rust-resistant hybrid, Pentad × Marquis (R.L. 730), was used. Unfortunately, this variety also proved to be somewhat resistant to kernel smudge, so that, in 1933, 1934 and 1935, the variety Pentad, which is resistant to stem rust in Manitoba but very susceptible to kernel smudge, was substituted.

The experiments from which the data shown in Table XI were taken were not specially designed to give information concerning kernel smudge. In the following years, however, a suitable plan of plot experiment was used. The seed was planted in rod rows, one foot apart, by means of a drill. After the plants had reached the shot blade stage of growth, pairs of rows, which were separated from pairs of control rows (undusted), were dusted with sulphur at the rate of 30 lb. per acre per application, at intervals of four days until the plants were practically mature. The undusted rows were protected from dust drift by three buffer rows. After the crop had been harvested and threshed, the percentage of discolored kernels, based on a count of 1000 kernels taken at random from the grain of each row, was calculated. The results of these tests, showing the percentage of smudged kernels from dusted and undusted plots of Pentad wheat, are given in Table XII.

TABLE XII

THE EFFECT OF DUSTING GROWING PLANTS OF PENTAD WHEAT WITH SULPHUR ON THE DEVELOPMENT OF KERNEL SMUDGE OF WHEAT DURING THE FOUR YEARS 1932 TO 1935

Treatment	Percentage of smudged kernels			
	1932	1933	1934	1935
Dusted	2.4	24.1	48.5	91.9
Undusted	3.3	27.3	51.5	91.0
Difference	0.9	3.2	3.0	− 0.9
Value of t*	3.07	4.24	3.53	1.79

* 5% point for value of t = 1.95.

Table XII shows that dusting the growing plants with sulphur reduced the disease by a significant degree in 1932, 1933, and 1934, but not in 1935. In no year, however, was the reduction sufficient to justify the use of this method of control.

THE EFFECT OF SEED TREATMENT

During the years 1932 to 1935 laboratory and greenhouse tests were made to determine the effect, on the control of seedling blight and root rot in the seedlings, of disinfecting seed infected with kernel smudge fungi with various chemicals. In these tests, mercuric bichloride steep, Semesan steep, copper carbonate dust, Ceresan and New Improved Ceresan were used. The results with seed produced in 1932, 1933, and 1934 were mostly negative, or at least inconclusive. That is to say, seed treatment did not give an increase in seedling emergence or an appreciable reduction in the amount of root rot. The reason for this seemed to be that *Alternaria* was the dominant fungus in the seed produced in these years, so that little or no disease developed in the seedlings, even in the controls.

TABLE XIII

EFFECT OF TREATING PENTAD WHEAT SEED, NATURALLY INFECTED WITH KERNEL SMUDGE IN 1935, WITH NEW IMPROVED CERESAN, LEYTOSAN, AND COPPER CARBONATE DUSTS, ON PLANT EMERGENCE, INCIDENCE OF ROOT ROT, AND YIELD IN 1936

Analyses of Variance

Data	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	5% point
Plant emergence	Replicates	5	726.89	145.37		
	Fungicides	2	13676.44	6838.22	210.47	3.88
	Error (a)	12	389.95	32.49		
	Seed (dusted and undusted)	1	8040.11	8040.11	137.43	4.54
	Error (b)	15	877.50	58.50		
	Total	35	23710.89			
Disease rating	Replicates	5	445.83	89.16		
	Fungicides	2	7340.41	3670.20	272.27	3.88
	Error (a)	12	161.78	13.48		
	Seed (dusted and undusted)	1	4489.00	4489.00	111.61	4.54
	Error (b)	15	603.36	40.22		
	Total	35	13040.38			
Yield	Replicates	5	32.53	6.50		
	Fungicides	2	68.96	34.48	7.32	3.88
	Error (a)	12	56.52	4.71		
	Seed (dusted and undusted)	1	218.05	218.05	24.09	4.54
	Error (b)	15	135.77	9.05		
	Total	35	511.83			

Summary of Results

Data	Seed treatment	New Improved Ceresan	Leytosan	Copper carbonate	Standard error
Emergence (per cent)	Dusted	84	89	29	±2.33
	Undusted	38	39	38	±2.33
	Difference	+46	+50	− 9	
	Standard error	± 3.12	± 3.12	± 3.12	
Disease rating*	Dusted	40	37	81	±1.50
	Undusted	75	74	76	±1.50
	Difference	−35	−37	+ 5	
	Standard error	± 2.58	± 2.58	± 2.58	
Yield (bu. per acre)	Dusted	19.2	17.9	14.5	±0.89
	Undusted	12.6	10.6	13.7	±0.89
	Difference	+ 6.6	+ 7.3	+ 0.8	
	Standard error	± 1.23	± 1.23	± 1.23	

* *Computed on the basis of number of seeds sown; highest numerical rating given for non-emergence.*

In the autumn of 1935, when seed infected and discolored by *H. sativum* was available, greenhouse tests with the diseased seed showed that disinfection with two commercial dusts, New Improved Ceresan and Leytosan, resulted in a marked control of seedling blight. From these tests it appeared that both fungicides were lethel to the mycelium within the seed coats, as well as to surface-borne spores.

In 1936, a comprehensive field experiment was made in which three dusts (New Improved Ceresan, Leytosan, and copper carbonate) were used to treat discolored seed of Pentad wheat from the 1935 crop. Table XIII gives the complete analyses of variance of the experimental data, and presents a summary table showing the effect of the treatments on seedling emergence, severity of the root-rot disease, and on yield. The data show that, while treatment with copper carbonate was not beneficial, treatment with New Improved Ceresan and Leytosan improved the stand, reduced root rot and increased yield. An increase of about seven bushels per acre was obtained in 1936 as the result of treating Pentad seed, infected with *H. sativum*, with the two organic mercurials.

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LABYRINTHULA ON PACIFIC COAST EEL-GRASS[1]

BY EDWARD LORRAINE YOUNG III[2]

Abstract

The labyrinthulan parasite causing the disease of the Atlantic coast *Zostera marina* L. is reported on *Z. marina* from Departure Bay, Nanaimo, B.C. The gross and histological appearance of infected Pacific coast eel-grass are described and are found to be similar to those of the Atlantic coast grass and the parasites, in each case, are found to be identical.

Introduction

One of the *Labyrinthulae* was shown to be the causative agent of the *Zostera marina* wastage in the Atlantic Ocean by Dr. Charles E. Renn in 1934 (1, 2). He studied specimens of infected eel-grass from many parts of the American and European coasts and found that both the symptoms of the disease and the labyrinthulan parasite associated with them remained constant. The leaves of infected beds are readily identified by their splotched and darkly streaked appearance and they are sloughed off shortly after the disintegration of the greater part of their green tissues. The discoloration is due to the disruption of both the epidermal and mesophyll cells. Death is caused apparently by the destruction of the food synthesizing mechanism, as the stems and roots remain unaffected. Dr. Renn examined grass from Departure Bay in September 1934 and again in the early part of 1936, but found neither symptoms of the disease nor *Labyrinthula*. Another shipment of grass from the Pacific coast in June, 1937, was also negative.

Discovery of *Labyrinthula* on Pacific Coast Eel-grass

Collections of *Zostera marina* L. were made at the Departure Bay Biological Station, Nanaimo, B.C. in September, 1936, by Dr. H. T. Güssow and again in July, 1937, by Dr. W. A. Clemens, and were shipped to the writer for examination by Dr. Irene Mounce. *Labyrinthula* was demonstrated conclusively in both shipments. The specimens of grass were preponderantly of the variety *latifolia*. From gross external appearance the disease manifests itself by the same brown and blackened splotched areas on the blades as it does in the Atlantic Ocean infestation. For study the leaves, preserved in a formalin-acetic-alcohol fixative, were embedded in paraffin, sectioned at 10 microns, and stained with a modification of the Staughton technique for

[1] *Manuscript received September 25, 1937.*

[2] *Formerly student, Biological Laboratories, Harvard University, Cambridge, Mass., U.S.A. Address at present 249 Dean Road, Brookline, Mass.*

wood (safranin, carbol-thionin, orange G). With this stain the tissues are easily differentiated: the diseased parts yellow to orange, the healthy blue to purple, and *Labyrinthula* a gray blue. The chloroplasts also stain blue but are more purple and brilliant than the parasite.

Histologically the picture seen here is identical with that observed in Atlantic Ocean *Z. marina*. The healthy normal tissue is bordered by diseased cells. The contents of these latter cells are in various stages of dissolution: the chloroplasts are disrupted; the protoplasm is flocculated and clumped either at the periphery or in the centre of the cell; the cell walls are irregular. Many cells are completely emptied of their contents while others are filled with secondary inclusions such as resins. Often the mesophyll air spaces are occluded by these ergastic substances.

The parasite is most abundant just ahead of the badly diseased tissue either in recently invaded cells or just beyond in apparently still healthy areas. Scattered spindles (cell bodies) of the parasite and resting bodies are found in areas of older infection. The parasite is most clearly demonstrated in the mesophyll air spaces.

The organism present in western eel-grass appears to be identical with that found on the Atlantic grass which has been identified by Young as *Labyrinthula macrocystis* Cienkowski. The cell bodies or spindles are fusiform and average $10 \times 3\mu$. A central vesicular nucleus and nucleolus are visible. These spindles are joined by a filamentous net-track which extends from cell to cell. On this network the spindles glide, boring through the cell walls probably by enzymatic action. The resting bodies are spherical and 6–10μ in diameter. In sum, the pathological picture presented by the *Zostera marina* from Departure Bay is similar in all respects to that of grass from any part of the Atlantic seaboard.

Discussion

It is difficult either to account for the presence of *Labyrinthula* in Pacific coast eel-grass or to foretell what it may mean. The sudden appearance of the disease on the Atlantic coast has not been explained satisfactorily as yet, and so it is equally hard to account for its sudden occurrence at Nanaimo. Oceanographic and climatic data supplied through the courtesy of Dr. W. A. Clemens, Director of the Biological Station at Departure Bay, are being studied in an attempt to find some one or more environmental factors which have changed sufficiently and universally enough to account for its sudden rise. Temperature changes appear inadequate since the water at Departure Bay has increased in mean yearly temperature but 0.8° C. in the last three years (10.9° to 11.7° C.). It may be of more significance that the salinity has increased as it has been found along the Atlantic coast that grass in sheltered areas of reduced salinity appeared more resistant to the disease. For June, 1934 to March 1935, the mean chlorinity percentage at Departure Bay was 13.69; for the next year 14.30; and for this last year 14.72.

On the other hand Renn and Lynch examined plantations of Pacific grass in Great Bay, Long Island, in early June, 1936. These beds were in seed and were beginning to waste away, but without exhibiting the black streaks or splotches characteristic of the labyrinthulan disease, and no ***Labyrinthulae*** were found on examination. Whether the eel-grass on the Pacific coast is not very susceptible to the disease, whether environmental conditions are not such as to favor the development of an epidemic, whether the parasite may remain endemic in Departure Bay, or whether the western eel-grass too may be wiped out remains at present a matter of conjecture.

Acknowledgments

The author is much indebted to Dr. Irene Mounce for supplying the grass from Departure Bay and for her interest and help in preparing this paper; to Dr. C. E. Renn, Dr. W. A. Clemens, and Dr. H. T. Güssow for the information and specimens which they furnished.

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YELLOW BLOTCH-CURL: A NEW VIRUS DISEASE OF THE RED RASPBERRY IN ONTARIO[1]

By G. C. Chamberlain[2]

Abstract

This paper reports the results of an investigation into a condition of the Cuthbert red raspberry which is quite distinct from any previously described disease. *The disease, termed yellow blotch-curl, is characterized principally* by a loose type of curling and pale chlorotic foliage which sometimes shows a yellow blotching and ring spot. The disease has been transmitted by patch-*grafting, which indicates that it is of the virus type. It has been transmitted to* Cuthbert, Viking, Latham, Herbert, Chief, and Lloyd George varieties, and different reactions have been noted and described.

From these experiments it is concluded that "yellow blotch-curl" is distinct from mosaic and leaf curl.

Introduction

In 1935 a general investigation of the virus diseases of the red raspberry was undertaken in order to compare varietal reactions to the mosaics and leaf curl, and to study the possibility of virus infection in any plants showing unusual symptoms. During the latter phase of this work, two new virus diseases were encountered, one of which is dealt with in the present paper. This was found in a Cuthbert plantation where a number of plants at one end of the nursery rows showed a decided lack of vigor accompanied by an unusual arching and curling of the foliage. This condition, which had been observed in other plantings, has hitherto been ascribed to some unfavorable soil condition. The possibility of virus infection, however, was suggested by the presence on one of the canes of several basal leaflets exhibiting distinct yellow blotches or spots, some of which were in the form of rings. This cane was brought to the laboratory for use as a source of scion material for transmission experiments by grafting. These experiments have been successful in determining the cause to be virus infection. Because of the character of the symptoms, the name "yellow blotch-curl" is suggested.

Occurrence

Undoubtedly this disease has been present in Ontario for a number of years, although it was not recognized as such until 1935. It has been observed repeatedly as an important factor in the variety Cuthbert, but has not been recognized in other varieties, except as the result of grafting. In 1937, all plantings visited were critically inspected, with the result that in five of seven nurseries and six of eleven commercial plantings the disease could be readily found. In one instance, as a result of investigating a complaint regarding the failure of a five-year-old three-acre plantation it was found that 90% of the stools were seriously affected. This plantation, though in good fertile gravelly

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Contribution No. 536 from the Division of Botany, Experimental Farms Branch, Dominion Department of Agriculture, Ottawa, Ontario.
[2] *Plant Pathologist, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario.*

soil and given excellent care, had steadily declined in production until it was no longer profitable and was destroyed. Since there was but a trace of mosaic and leaf curl present and other factors were favorable for production, the failure could only be ascribed to the effect of yellow blotch-curl disease.

Symptoms on Cuthbert in the Field

A noticeable effect of this disease is the reduction in number of canes and a remarkable dwarfing and stunting of growth. Affected stools stand out in contrast to normal ones and appear thin, spindly and lacking vigor. The appearance of such stools is very suggestive of the effect expected from a depleted soil or lack of drainage. The canes exhibit an erect, stiff type of growth and bear pale, chlorotic lustreless foliage. Owing to a shortening of internodes, particularly in the apical regions of the cane, the leaflets appear to cluster. This clustering is accentuated by a loose type of curling in which the leaves become arched and the tips curl down and inwards (Plate I, Fig. 1). The leaf texture also becomes such that a noticeable rattle is heard when the canes are shaken.

While the above symptoms are characteristic and commonly encountered, canes may also be found on which a few of the older basal leaflets exhibit a coarse, yellow blotching and spotting, often as definite ring spots. Frequently, however, these symptoms do not occur.

Experimental Work

Materials and Methods

The patch-grafting method, used for transmitting the virus, consists of replacing a patch of bark from a stock plant with a similar one from a scion plant. A special set of razor-blade knives, originated by and obtained from W. A. Roach (6) was used to make patches of equal size. After the graft was made, it was wrapped for a week with a crepe rubber bandage known as Sterilastic. It was later found desirable to cover the wound first with a piece of waxed paper to prevent the bandage adhering to the patch. The varieties used for grafting included Cuthbert, Viking, Latham, Herbert, Chief and Lloyd George, selected because of their apparent differences in regard to klendusity and susceptibility to mosaic (5). With the exception of Latham, which were specially selected from known healthy stools, all the plants were of virus-free clonal origin and were set in the field eight feet apart each way and received good cultural attention. Care was taken to see that the suckers were confined to the immediate vicinity of the parent plant. The grafts were generally placed low on the canes, although any area where the cane was firm enough not to break at the cut proved satisfactory.

The results of these transmission experiments carried on over a period of three years, clearly show that yellow blotch-curl has been repeatedly transmitted by patch grafting and that it can be passed to varieties other than Cuthbert, on which it was first found. On the other hand, attempts to transmit the disease to black raspberries have so far resulted in failure.

The first indication of transmission was noted September 21, 1935, on one of the two Cuthbert canes grafted July 12. A clustering and curling of the leaflets accompanied by a coarse yellow blotchiness that appeared at the tip of a late lateral were similar to the symptoms on the scion cane. By the following spring, both grafted stools were systemically infected and showed identical symptoms typical of those noted in the field. These two stools therefore were used as sources of scion material for many of the later grafts which verified the transmissibility and distinctive character of this disease.

TABLE I

RESULTS OF EXPERIMENTS TO TRANSMIT YELLOW BLOTCH-CURL BY PATCH GRAFTING, 1935–37

Affected scion	Healthy stock	Date	No. of grafts	Transmission	
				Positive	Negative
Cuthbert*	Viking	12.7.35	1	0	1
Cuthbert*	Cuthbert	12.7.35	2	2	0
Cuthbert	Cuthbert	2.6.36	2	2	0
Cuthbert	Viking	2.6.36	3	3	0
Cuthbert	Latham	12.6.36	8	7	1
Cuthbert	Viking	3.7.36	2	0	2
Cuthbert	Cuthbert	3.7.36	2	1	1
Cuthbert	Chief	3.7.36	2	2	0
Cuthbert	Viking	21.8.36	3	3	0
Cuthbert*	Viking	21.8.36	2	2	0
Cuthbert	Cuthbert	21.8.36	3	2	1
Cuthbert	Latham	21.8.36	4	3	1
Cuthbert	L. George	21.8.36	2	1	1
Cuthbert	Herbert	21.8.36	3	3	0
Cuthbert	Viking	22.9.36	3	0	3
Cuthbert	Bl. Raspberry	11.6.37	9	0	9
Latham	Viking	25.6.37	2	2	0
Viking	Viking	25.6.37	5	4	1
Cuthbert	Viking	25.6.37	4	4	0
Latham	Cuthbert	25.6.37	2	1	1
Latham	Cuthbert	25.6.37	4	3	1
Cuthbert	Cuthbert	25.6.37	4	4	0
Cuthbert	Viking	7.7.37	5	4	1
Chief	Viking	7.7.37	2	0	2
Cuthbert	Cuthbert	7.7.37	4	3	1
Cuthbert*	Viking	14.7.37	4	0	4
Cuthbert*	Viking	17.7.37	4	1	3
Cuthbert*	Cuthbert	17.7.37	4	3	1
Cuthbert*	Viking	6.8.37	3	0	3
Cuthbert*	Cuthbert	6.8.37	3	0	3
Cuthbert*	Cuthbert	7.8.37	4	0	4
Cuthbert*	Viking	7.8.37	4	0	4
Totals			109	60	49

* *Scion material suspected of being infected with yellow blotch-curl.*

Yellow blotch-curl has been transmitted not only from Cuthbert to Cuthbert, but also from Cuthbert to Latham, Herbert, Chief, Lloyd George and Viking. It has been transferred back to Cuthbert from Latham and also

TABLE II

SUMMARY OF EXPERIMENTS ON VARIETAL REACTION OF RASPBERRIES TO YELLOW BLOTCH-CURL

	Mosaic			Yellow blotch-curl							
					Symptom expression				Effect on growth		
Variety	Klendusity	Susceptibility	Degree of symptom-expression	Effect or reaction	Stunting effect	Yellow blotch	Ring spot	Curling	Suckers	Vigor	Remarks
Latham	++	+++	Masked	++++	+++	++++	0	+++	0 – +	Poor	Cane died
Herbert	+++	+++	Pronounced	++++	+++	+++	0	+++	0 – +	Poor	Cane died
Chief	+++	+	Highly masked	+	+	+	+	++	++	Fair	Weak cane growth
L. George	+++	++	Fairly pronounced	+++	+++	++	0	+++	+	Poor	Cane died
Viking	+	+++	Pronounced	+++	+++	++++	++	+++	+	Poor	Weakened cane growth
Cuthbert	+	+++	Pronounced	++	++	++++	+++	+++	+++	Fair	Cane growth reduced

The + sign is used to denote the following: + low or slight; ++ moderate; +++ high or severe; ++++ very severe.
Under "Effect on growth" of suckers: 0 absent; + very limited; ++ limited; +++ average production.

from Viking to Viking. As with other virus diseases, different varietal reactions to yellow blotch-curl have been noted. This is indicated in Table II.

It is evident from Table II that the six varieties may be divided into three main groups according to their reaction to yellow blotch-curl infection. Latham and Herbert proved distinctly susceptible, the fruiting canes dying after a weak, stunted growth of laterals bearing small, tightly-curled leaves (Plate I, Fig. 2). In the majority of instances the stools were completely killed and suckers either failed to appear or died following a poor stunted growth. Chief and Cuthbert form a group, far less seriously affected, especially as regards the stooling ability of the plants. The fruiting canes showed the usual delay in foliation and a general weakened growth of laterals, and while the sucker production was reduced especially, in Chief, which is normally prolific in this regard, still a number developed and made fair growth. Lloyd George and Viking may be placed in an intermediate group in degree of susceptibility, even though the effect on the growth of the stool was drastic. The Lloyd George fruiting canes died prematurely, but unlike the Latham and Herbert, the stool produced a limited number of suckers which continued to produce a weak growth. The Viking canes in most cases produced a weak growth of fruiting laterals (Plate II, Fig. 2) and a few worthless, small, unevenly ripened and misshapen berries. New cane production was *nil* or very limited and poor in vigor and growth.

The chlorotic or yellow blotch and spot symptoms were always more or less evident on grafted plants. It was, however, found to vary in occurrence and definition on the different varieties. On Latham, Viking and Cuthbert, for instance, it was not only more common but more pronounced (Plate II, Figs. 1 and 3). Very often the blotching was extensive, involving the entire lamina; in other cases it was more limited and confined to areas along the main veins or at the leaf margin. Sometimes it occurred as definite spots of varying size either singly or in groups. There was no mottling associated with this symptom nor was it accompanied by blistering or puckering of the leaf surface, although an asymmetrical distortion sometimes followed the occurrence of the blotches about main veins or at the leaf margin. In Cuthbert the blotching occasionally took the form of a yellow pattern. In general, these symptoms were confined to the older or early formed leaves, although at times they would appear in distinct zones similar to mosaic mottling. On both Lloyd George and Chief this symptom was suppressed. In Chief, particularly, the blotching was indistinct and appeared more as a paling of color and without definite character. The ring spots associated with the blotch symptom appeared on only three varieties, commonly on Cuthbert, infrequently on Viking, and only occasionally on Chief. On the latter, the rings were faint and bordered with a pale green, rather than a pronounced yellow as in the others.

The curling of the foliage was a symptom character common to all the varieties. On the fruiting canes of Latham, Herbert and Viking, a decided

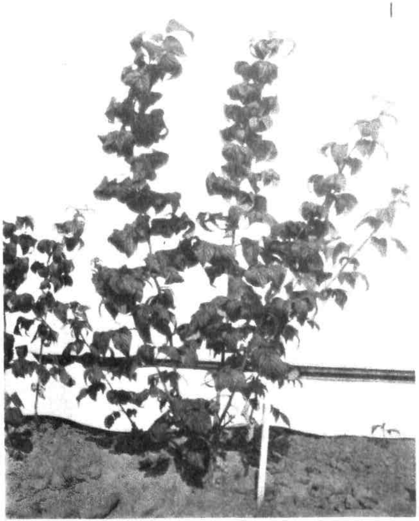

FIG. 1. *A Cuthbert stool affected with yellow blotch-curl. Note clustering and curling of leaflets. Grafted August 21, 1936. Photographed August 26, 1937.* FIG. 2. *Three Latham fruiting canes affected with yellow blotch-curl in varying degrees. A, slightly affected; B, disease not entirely systemic, all laterals on one side of cane seriously affected; C, severe effect; the stunting of laterals in C is very drastic. Leaves show extensive yellow blotches. From stools grafted June 12, 1936. Photographed June 3, 1937.*

FIG. 1. *Leaflets from a Viking cane affected with yellow blotch-curl showing blotching and spotting of the leaf.* FIG. 2. *The effect of yellow blotch-curl on fruiting cane of Viking, centre; compared with mosaic, left, and healthy cane, right. Grafted August 21, 1936. Photographed June 3, 1937.* FIG. 3. *Leaflets of Cuthbert showing blotching, spotting and ring spot symptom of yellow blotch-curl common on grafted plants. From plant grafted June 2, 1936. Photographed September 18, 1936.*

curl accompanied by dwarfing of foliage developed. On the new canes of these varieties it was less drastic, but it was characteristic for the growth to cease when the tips became curled. On the other hand the new canes of the other varieties continued to grow and showed a clustering effect of leaflets and a loose curling of the foliage, the symptom commonly encountered in the field (Plate I, Fig. 1).

Discussion

In all cases where transmission occurred it was noted that infection was not systemic until the second year. Symptoms appeared first, not on the central leader of the grafted cane, but on one or more lateral branches and most frequently on those arising nearest the graft. With varieties such as Latham, which form few branches, the first symptoms show on a few of the suckers and not on the original grafted cane except as the disease becomes systemic the following year. This was also found to be true in cases where mosaic scion material was used for grafting.

June or early July proved to be the most favorable time for grafting. At this time growth is succulent, with the result that scion and stock patches strip clean and are easily handled. In addition, the results obtained indicate that the incubation period for yellow blotch-curl may vary considerably and is quite lengthy, and, therefore, unless grafts are made in June or early July, symptoms do not appear on the current season's growth. There was also evidence to indicate that the incubation period may be shortened if vigorous, rapidly growing plants are inoculated. This was demonstrated in 1937 when a more favorable growing season made earlier grafting possible. In one graft a period of 45 days elapsed between the time of grafting and symptom expression, compared to more than 70 days in 1936.

The relation of early grafting to symptom expression in the current season may be mentioned in connection with the grafts made in August, 1937. These grafts are recorded in Table I as negative so far as transmission is concerned. However, the results are as yet incomplete, as previous experience suggests the grafts were made too late in the season for symptoms to appear.

The two virus diseases, mosaic and leaf curl, have been of importance in Ontario for the past 25 years. Mosaic is manifested by a general mottling, accompanied by blistering and puckering of the foliage. The symptoms of leaf curl are a severe tight curling of the foliage which is dark green and appears greasy (3). Both these diseases have been transmitted by patch grafting with resulting symptoms typical for each disease. The symptoms as described above for yellow blotch-curl are therefore sufficiently distinct from either mosaic or leaf curl to warrant considering yellow blotch-curl as a new virus disease. While no definite survey has as yet been made to determine the importance of this disease, it is felt from a limited experience that it may be of more importance than is at present realized.

Acknowledgment

The author wishes to express his indebtedness to Dr. G. H. Berkeley, Senior Officer-in-charge of the Dominion Laboratory of Plant Pathology at St. Catharines, Ontario, for his direction and help during the course of the work.

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STUDIES ON FOOT AND ROOT ROT OF WHEAT

V. THE RELATION OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM NUTRITION TO THE FOOT- AND ROOT-ROT DISEASE OF WHEAT CAUSED BY *HELMINTHOSPORIUM SATIVUM* P. K. & B.[1]

By W. C. Broadfoot[2] and L. E. Tyner[3]

Abstract

The effect of different amounts of phosphorus, potassium, nitrogen, and calcium upon the development of the foot-rot disease of wheat caused by *Helminthosporium sativum* P. K. & B. was studied in the greenhouse. The wheat grains were planted in sterilized pure quartz sand to which the necessary nutrients and spore suspension of the pathogen were added. The experiments were maintained under aseptic conditions during the first ten days. The disease increased when the ionic concentration of potassium, nitrogen, and calcium was decreased below that of the complete nutrient solution, but no significant reduction of the disease was observed when the concentrations of all of the elements, including phosphorus, were increased above those in the complete nutrient solution. Apparently extremely small concentrations of phosphorus had no effect on the disease one way or the other. These conclusions apply to the disease on the seedling stage of wheat.

Of the many food and other growth factors which contribute to the general health of the wheat plant, probably none is more important than mineral nutrition. The symptoms of nitrogen, phosphorus and potassium starvation of this plant, and the remedy for it, are well known and fully described in a number of books. An extensive review of the literature relating to the factors affecting the absorption of these essential elements by plants has been made by Hoagland (2). There is also the direct role of a deficiency of certain minor elements in producing pathological conditions, which has been discussed by Jacks and Scherbatoff (3).

In addition to the direct effect on plant growth of the minerals referred to above, a deficiency or excess of one or more of these might render a host more susceptible to certain disease-producing micro-organisms than it would otherwise be. This premise would seem to have special application to the foot- and root-rot diseases of cereals, since both host and pathogen must develop in the same soil habitat where the metabolism of each is undoubtedly affected by the kind of minerals available. No reference, however, has been found in the literature concerning the effect of the absorption of varying amounts of nitrogen, phosphorus, potassium, and calcium on the ability of the wheat plant to withstand infection and injury by *Helminthosporium sativum* P. K. & B.

Since our early field experiments on mineral nutrition gave extremely variable results, it became necessary to transfer the study to the greenhouse,

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[2] *Plant Pathologist, Dominion Laboratory of Plant Pathology, Edmonton, Canada.*
[3] *Plant Disease Investigator, Dominion Laboratory of Plant Pathology, Edmonton, Canada.*

where the chemical and other usual environmental factors could be better controlled, and also where a more precise experimental technique could be employed. The present greenhouse investigation has been principally concerned with the nutrition of the wheat plant as a factor in its pre-disposition to attack by *H. sativum*. It has involved a study of the development of the foot and root rot of wheat caused by this pathogen in known ionic concentrations of nitrogen, phosphorus, potassium, and calcium as added to a silica sand substrate. Observations were also made on the growth of the pathogen itself in pure culture solutions to which the minerals mentioned were added.

Materials and Methods

The nutrient solutions employed were varied according to need from basic formulas which were kindly furnished by Professor D. R. Hoagland, of the University of California. His complete solution (Table I) was used as a basis for comparison in all experments. The required chemicals, in C.P. form, were added to wide-mouth, one-litre flasks, each containing one-half kilogram of pure silica sand, obtained from Ottawa, Illinois. The flasks, with cotton plugs, were autoclaved at 20 lb. pressure for 6 hr. Each flask was aseptically seeded with 30 grains of Elite Marquis wheat, which had been previously soaked in water for 3 hr. at 20° C. and surface disinfected in 1-1000 $HgCl_2$ for 20 min. The planted seeds were drenched with a spore suspension of a virulent one-month-old culture of *H. sativum*, and were then covered with 250 cc. of sterilized silica sand. In the control series, the spore suspension was replaced by an equal volume of sterile distilled water. The flasks were weighed at weekly intervals and the sand was maintained at 70% of its moisture-holding capacity by adding distilled water containing 1 cc. per litre of 0.5% ferric tartrate. At the end of 40 days, the number of plants, the height, and the infection rating were recorded. The plants were dried for 48 hr. in an oven at 90° C., and then weighed. The following values for infection were assigned: 0, clean; 1, trace; 2, light−; 3, light; 4, light+; 5, medium−; 6, medium; 7, medium+; 8, heavy−; 9, heavy; 10, heavy+. Four experiments for the silica sand series are reported, *viz.*: Nos. 1, 2, 3, and 8.

The results obtained from growing the pathogen in pure culture, in the same nutrient solutions as were used in the corresponding silica sand experiments, are presented separately. Other necessary details of technique will be supplied in conjunction with each experiment. The experimental data were tested by Fisher's (1) Analysis of Variance method, and the "F" test of Snedecor (4), to determine the significance of the differences observed between the various treatments.

Experimental Results

Experiment I

Effect of Omission and of Excess of P, K, N, and Ca

In this experiment the effect of the omission and also of the excess of phosphorus, potassium, nitrogen and calcium ions upon the development of disease

was investigated. The composition of the nutrient solutions is given in Table I. Each nutrient solution in both infested and control series was replicated ten times. This experiment was taken up on November 7, 1933.

An examination of the data in Table II shows that the F value for nutrients exceeded the 1% point for number, height, and infection rating in the infested series. It is quite evident that, for each of the nutrients, the plants in the infested series were shorter and lighter than the plants in the control series (see Plate I). Disease development was most severe in the solutions deficient

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS (CC. PER LITRE) EMPLOYED IN EXPERIMENT I

Nutrient solutions	$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4	K_2SO_4	$Ca(H_2PO_4)_2$	H_3PO_4	KCl	NH_4NO_3	$CaCl_2$
	M/1	*M*/1	*M*/1	*M*/1	M/.5	*M*/.01	M/1	*M*/1	M/1	*M*/1
1 H_2O (dist.)										
2 Complete	5	5	2	1						
3 Minus P	7.5		2		10					
4 Excess P	7.5		2	1		50	5			
5 Minus K	7.5		2			50				
6 Excess K	5	5	2	1	10			5		
7 Minus N			2		10	50				
8 Excess N	8	5	2			50			5	
9 Minus Ca		10	2	1						
10 Excess Ca	7.5		2		10	50				5

TABLE II

EFFECT OF THE OMISSION AND THE EXCESS OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM UPON THE NUMBER, HEIGHT, INFECTION RATING, AND WEIGHT, OF MARQUIS WHEAT SEEDLINGS GROWN IN SILICA SAND, INFESTED WITH *Helminthosporium sativum*

Nutrient solutions	*Number of plants*		*Height, cm.*		Infection rating	*Weight, mg.*	
	Infested series	Control	Infested series	Control		Infested series	Control
1 H_2O (dist.)	22.9 −	26.5	19.9	25.4 −	8.0 +	21.4	29.2
2 Complete	25.3	28.9	30.1	35.6	5.6	46.7	61.3
3 Minus P	27.9 +	26.9	30.0	36.1	4.4 −	56.3	58.6
4 Excess P	26.9	27.6	29.9	34.5 −	4.4 −	48.3	58.8
5 Minus K	23.3	22.9 −	16.0 −	19.1 −	8.8 +	24.0	21.3
6 Excess K	26.6	23.4 −	28.9	32.0 −	4.0 −	64.3	65.8
7 Minus N	28.0 +	28.3	20.1 −	24.0 −	6.4 +	35.0	36.7
8 Excess N	26.0	28.3	29.5	34.4 −	5.0	60.8	65.9
9 Minus Ca	24.7	24.6 −	18.4 −	21.6 −	8.8 +	22.3	27.4
10 Excess Ca	28.0 +	27.0	31.8 +	34.6 −	4.5 −	63.2	74.5
$2 \times \sqrt{2} \times$ S.E.	2.1	2.5	1.0	0.9	0.5		
"F" nutrients	6.24	5.66	307.34	512.67	108.99		
1% point	2.74	2.82	2.74	2.82	2.74		
"F" replicates	.78	.38	8.55	15.00	1.01		
5% point	2.06	2.25	2.06	2.25	2.06		

+ and − indicate that these values are significantly higher or lower than those for the complete solution.

in potassium, nitrogen and calcium. Although it would appear that disease development was less on the plants in the solutions with excess phosphorus, potassium, nitrogen and calcium than in the complete nutrient solution, quantitative data, such as height, suggest that there is no marked difference. The error due to the difficulty of washing out all of the silica sand from the roots vitiated the reliability of the data on the dry weight of the plants. It was apparent, from the growth of the plants obtained in the phosphorus-deficient series that sufficient phosphorus was provided by the seed or by impurities in either the C.P. chemicals or the silica sand used.

Experiment II

Effect of Deficiency and of Excess of P, K, N, and Ca

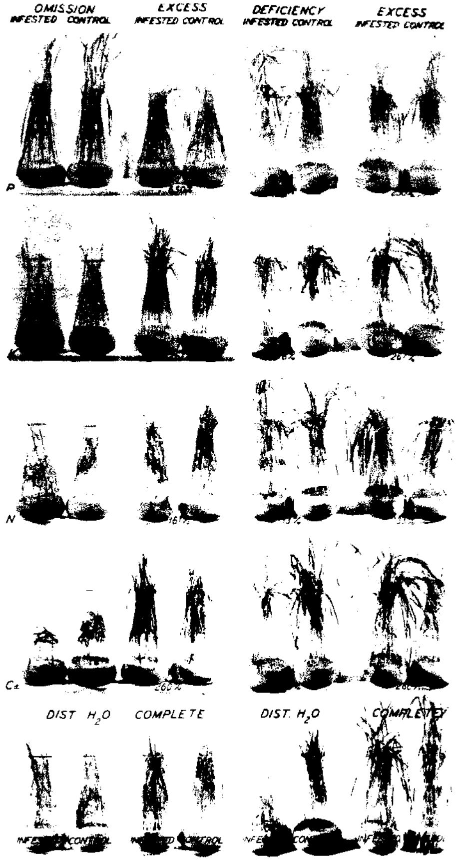
In Experiment I, very poor growth was obtained in the series where potassium, nitrogen and calcium ions were omitted from the solutions. Therefore, in this experiment the effect of a deficient amount of phosphorus, potassium, nitrogen and calcium ions, and also of an excess of them, upon the development of disease was observed. The composition of the nutrient solutions is given in Table III. Each nutrient solution was replicated ten times in the infested series, and five times in the control series. This experiment was taken up on February 9, 1934.

As in Experiment I, the F value of the effect of the nutrients exceeded the 1% point for number, height, and infection rating in the series containing the pathogen, and for height in the series where the pathogen was omitted. The plants in the infested series were shorter than those with the same nutrient solution in the control series (Table IV, Plate I). Although the development of disease was more severe in the potassium-, nitrogen- and calcium-deficient series than in the excess series, it was, nevertheless, not as severe as it was in Experiment I, where these ions were entirely omitted. In other words, it appears that in the presence of a restricted supply of the ions, the incidence of the disease was more severe than in the complete culture, but not as severe as in the cultures from which they were omitted entirely.

TABLE III

Composition of nutrient solutions (cc. per litre) employed in Experiment II

Nutrient solutions	$Ca(NO_3)_2$	KNO_3	$MgSO_4$	KH_2PO_4	K_2SO_4	$Ca(H_2PO_4)_2$	H_3PO_4	KCl	NH_4NO_3	$CaCl_2$
	M/1	*M/1*	*M/1*	*M/.1*	*M/.5*	*M/.01*	*M/1*	*M/1*	*M/1*	*M/1*
1 H_2O (dist.)										
2 Complete	5	5	2	1						
3 Minus P	5		2		10					
4 Excess P	7.5		2	1		50	1			
5 8% K	7.5		2		1	50				
6 Excess K	2	5	2	1	10			5		
7 13% N	1		2		10	50				
8 Excess N	5	5	2			50			5	
9 20% Ca	1	10	2	1						
10 Excess Ca	7.5		2		10	50				5



Effect of the omission, deficiency, and excess of phosphorus, potassium, nitrogen, and calcium upon the development of foot rot of Marquis wheat seedlings grown in silica sand, infested with Helminthosporium sativum.

TABLE IV

EFFECT OF THE DEFICIENCY AND THE EXCESS OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM UPON THE NUMBER, HEIGHT, INFECTION RATING AND WEIGHT OF MARQUIS WHEAT SEEDLINGS GROWN IN SILICA SAND, INFESTED WITH *Helminthosporium sativum*

Nutrient solutions	Number of plants		Height, cm.		Infection rating	Weight, mg.	
	Infested series	Control	Infested series	Control		Infested series	Control
1 H_2O (dist.)	21.6 –	26.2 –	16.8 –	23.0 –	9.2 +	20.9	26.0
2 Complete	27.1	28.8	39.8	45.0	5.2	43.9	67.9
3 Minus P	27.7	28.2	38.1	44.0	5.4	59.2	71.7
4 Excess P	27.8	28.0	37.7 –	42.0	4.9	55.4	57.9
5 8% K	27.0	28.6	38.6	38.8 –	7.0 +	58.1	60.1
6 Excess K	24.9 –	26.8 –	34.5 –	42.8	4.8 –	47.2	51.5
7 13% N	25.8	28.4	32.9 –	43.0	5.8 +	48.8	51.4
8 Excess N	26.8	29.2	36.3 –	45.0	5.1	47.4	66.4
9 20% Ca	26.7	28.8	37.0 –	41.5 –	5.9 +	47.6	58.3
10 Excess Ca	26.0	28.2	40.3	44.0	4.6 –	61.2	58.9
$2 \times \sqrt{2} \times$ S.E.	1.9	1.7	.9	3.0	.4		
"F" nutrients	7.26	2.50	118.68	37.26	128.51		
1% point	2.74	3.07	2.74	3.07	2.74		
"F" replicates	1.43	.47	.65	.66	1.39		
5% point	2.06	2.64	2.06	2.64	2.06		

+ and – indicate that these values are significantly higher or lower than those for the complete solution.

EXPERIMENT III

Effect of Decreasing the Concentration of K, N, and Ca

The addition of small amounts of potassium, nitrogen and calcium ions in Experiment II reduced the disease markedly. Therefore, the effect of varying, within narrow limits, the concentration of these ions upon the development of the disease was studied in this experiment. The composition of the nutrient solutions is given in Table V. Each nutrient solution was replicated five times. This experiment was taken up on April 7, 1934.

The experimental data given in Table VI, and illustrated in Fig. 1, verified the results of Experiment II, which indicated the necessity of having at least minimum amounts of these three ions (K, N, and Ca). The F value for nutrients exceeded the 1% point for number, height, and infection rating. Thus, by decreasing the ionic concentration of K, N, and Ca the disease was increased, and also the height and weight of the plants were decreased.

EXPERIMENT VIII

Effect of Increasing the Concentration of P, K, N, and Ca

Since sub-optimal amounts of potassium, nitrogen and calcium materially affected the metabolism of the wheat plant, as expressed in the data given, it seemed that by increasing the ionic concentration, the disease might be reduced. Using the complete nutrient solution as a base, 2, 4, 6, 8 and 10 cc. per litre of a molar solution of NaH_2PO_4, KCl, NH_4Cl, and $CaCl_2$ was

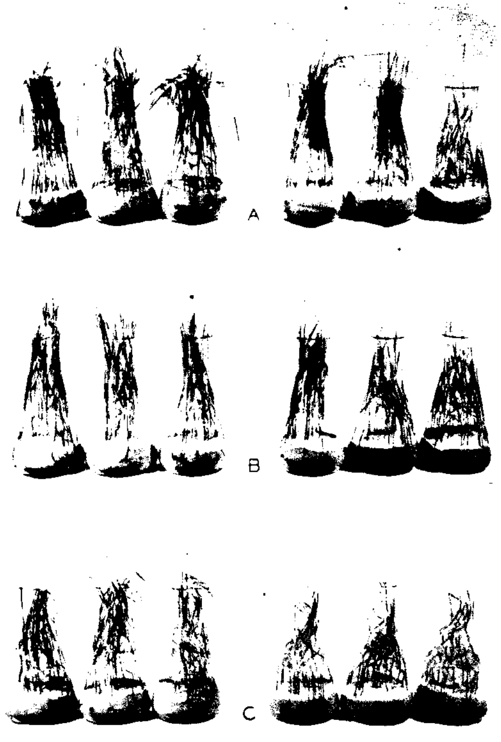


FIG. 1. *Effect of decreasing the ionic concentration of potassium, nitrogen, and calcium upon the development of foot rot of Marquis wheat seedlings grown in silica sand, infested with Helminthosporium sativum. Left to right: A—17% K, 13% K, 9% K, 4% K, 2% K, minus K. B—13% N, 10% N, 7% N, 3% N, 1% N, minus N. C—20% Ca, 15% Ca, 10% Ca, 5% Ca, 2% Ca, minus Ca.*

added to give nutrient solutions of increasing concentrations of phosphorus, potassium, nitrogen and calcium, respectively. Each nutrient was replicated five times. This experiment was taken up February 20, 1935.

The experimental results given in Table VII indicate that, according to the criteria used in these experiments, the increasing of the concentration of

TABLE V

COMPOSITION OF NUTRIENT SOLUTIONS (CC. PER LITRE) EMPLOYED IN EXPERIMENT III

Nutrient solutions	$Ca(NO_3)_2$ *M/1*	KNO_3 *M/1*	$MgSO_4$ *M/1*	KH_2PO_4 *M/1*	$Ca(H_2PO_4)_2$ *M/.01*	K_2SO_4 *M/.5*
1 H_2O (dist.)						
2 Complete	5	5	2	1		
3 17% K	7.5	1	2		50	
4 13% K	7.5	.75	2		50	
5 9% K	7.5	.50	2		50	
6 4% K	7.5	.25	2		50	
7 2% K	7.5	.10	2		50	
8 Minus K	7.5		2		50	
9 13% N	1.0		2		50	10
10 10% N	.75		2		50	10
11 7% N	.50		2		50	10
12 3% N	.25		2		50	10
13 1% N	.10		2		50	10
14 Minus N			2		50	10
15 20% Ca	1.0	10	2	1		
16 15% Ca	.75	10	2	1		
17 10% Ca	.50	10	2	1		
18 5% Ca	.25	10	2	1		
19 2% Ca	.10	10	2	1		
20 Minus Ca		10	2	1		

TABLE VI

EFFECT OF DECREASING THE IONIC CONCENTRATION OF POTASSIUM, NITROGEN AND CALCIUM UPON THE NUMBER, HEIGHT, INFECTION RATING, AND WEIGHT OF MARQUIS WHEAT SEEDLINGS GROWN IN SILICA SAND, INFESTED WITH *Helminthosporium sativum*

Nutrient solutions	Number of plants	Height, cm.	Infection rating	Weight, mg.
1 H_2O (dist.)	22.2−	18.1−	8.6+	36.0
2 Complete	28.6	30.1	5.2	125.2
3 17% K	28.0	31.1	5.7	118.6
4 13% K	26.8	32.4+	6.0+	131.3
5 9% K	27.8	29.3	6.3+	107.9
6 4% K	27.4	30.0	6.6+	75.9
7 2% K	26.4	26.4−	6.9+	65.9
8 Minus K	25.6−	19.8−	8.1+	40.6
9 13% N	26.2	28.5−	5.1	77.9
10 10% N	27.6	25.1−	5.8+	73.9
11 7% N	26.6	22.2−	6.4+	60.2
12 3% N	27.0	21.3−	6.9+	52.6
13 1% N	27.6	18.3−	7.5+	51.4
14 Minus N	27.0	18.8−	7.6+	47.4
15 20% Ca	26.2	26.2−	7.0+	64.9
16 15% Ca	25.4−	24.1−	6.8+	59.1
17 10% Ca	25.6−	20.2−	8.0+	42.2
18 5% Ca	26.6	17.8−	8.8+	46.6
19 2% Ca	26.2	16.0−	9.4+	42.7
20 Minus Ca	27.6	17.6−	9.6+	47.1
$2 \times \sqrt{2} \times$ S.E.	2.6	1.5	.6	
"F" Nutrients	2.26	141.98	41.82	
1% point	2.03	2.03	2.03	
"F" Replicates	1.50	2.77	3.04	
5% point	2.49	2.49	2.49	

\+ *and* − *indicate that these values are significantly higher or lower than those for the complete solution.*

TABLE VII

EFFECT OF INCREASING THE IONIC CONCENTRATION OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM UPON THE NUMBER, HEIGHT, INFECTION RATING, AND WEIGHT OF MARQUIS WHEAT SEEDLINGS GROWN IN SILICA SAND, INFESTED WITH *Helminthosporium sativum*

Nutrient solutions	Number of plants	Height, cm.	Infection rating	Weight, mg.
1 H_2O (dist.)	27.6	18.3–	8.9+	23.2
2 Complete	28.0	32.1	4.6	72.1
3 300% P	27.4	30.8	4.8	59.1
4 500% P	28.8	31.8	4.1–	57.6
5 700% P	28.8	31.1	4.2	54.2
6 900% P	26.8	29.3–	4.1–	50.0
7 1100% P	27.6	26.1–	5.2+	41.3
8 133% K	27.6	31.5	4.8	87.7
9 167% K	27.6	31.3	4.2	85.5
10 200% K	28.2	31.3	4.3	85.1
11 233% K	28.6	29.9	4.4	71.3
12 267% K	25.4–	28.8–	4.1–	78.0
13 113% N	28.0	33.2	4.5	70.7
14 127% N	28.6	28.4–	4.6	57.5
15 140% N	28.2	29.9	4.0–	57.5
16 153% N	28.8	28.5–	4.3	55.6
17 167% N	25.4–	27.5–	4.5	55.9
18 140% Ca	28.0	30.8	4.5	82.9
19 180% Ca	26.8	30.7	4.3	83.6
20 220% Ca	26.6	28.8–	4.3	68.4
21 260% Ca	26.4	26.3–	4.6	59.1
22 300% Ca	26.0	24.3–	4.8	48.5
$2 \times \sqrt{2} \times$ S.E	2.1	2.5	0.5	
"F" Nutrients	2.27	13.44	38.41	
1% point	2.03	2.03	2.03	
"F" Replicates	1.41	.57	.10	
5% point	2.49	2.49	2.49	

+ and – indicate that these values are significantly higher or lower than those for the complete solution.

phosphorus, potassium, nitrogen and calcium above the concentration of these ions in the complete solution did not appreciably reduce the development of the disease. However, it is recognized that the complete solution is not necessarily of such concentration as to give maximum growth. If the concentration had been increased slightly above that for maximum growth, it is possible there would have been an effect on disease development. Again, the F value for nutrients exceeded the 1% point for number, height, and infection rating.

Studies with Helminthosporium sativum in Pure Culture

The purpose of this study was to secure additional information concerning the effect of the various concentrations of phosphorus, potassium, nitrogen and calcium on the vegetative growth of *H. sativum*. All nutrient solutions used for the silica sand series were tested, the only difference being that a 2% dextrose solution was added to each formula. The tests were run simultaneously with those made in the silica sand (Expts. I, II, III and VIII).

The media thus prepared were distributed in 125-cc. quantities in 200-cc. Erlenmeyer flasks, and sterilized. Four flasks of each medium were inoculated with a 2-mm. loop of a spore suspension of the pathogen, and incubated at room temperature. At the end of 40 days, the mycelial mats were filtered off, dried at 90° C. for 48 hr., and weighed.

The results, which are given in Table VIII, indicate that the growth of *H. sativum* was seriously decreased only where there was either an omission or an excess of nitrogen, and also in the non-nutrient solution. This was particularly evident where there was an excess of nitrogen in the ammonium form, as in Solution No. 8 in Experiments I and II, and also in Solutions Nos. 13 to 17, inclusive, in Experiment VIII. Obviously nitrogen in the nitrate form was very important as a food element. Thus, of the four elements

TABLE VIII

EFFECT OF VARYING THE IONIC CONCENTRATION* OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM UPON THE DRY WEIGHT OF THE VEGETATIVE GROWTH OF *Helminthosporium sativum* IN PURE CULTURE

Omission and excess		Deficiency and excess		Decreasing amounts		Increasing amounts	
Nusrient solutions	Weight, mg.	Nutrient solutions	Weight, mg.	Nutrient solutions	Weight, mg.	Nutrient solutions	Weight, mg.
1	28.5	1	23.0	1	37.5	1	35.8
2	506.8	2	570.2	2	570.3	2	532.0
3	435.8	3	515.3	3	701.3	3	480.0
4	444.3	4	658.3	4	656.7	4	474.8
5	344.2	5	697.7	5	667.5	5	407.2
6	335.8	6	689.0	6	681.7	6	404.8
7	48.3	7	733.7	7	683.0	7	382.0
8	94.7	8	113.3	8	128.5	8	566.7
9	351.0	9	558.3	9	683.7	9	664.7
10	558.0	10	638.0	10	542.7	10	549.3
				11	450.0	11	645.3
				12	231.8	12	566.5
				13	167.0	13	218.0
				14	45.1	14	264.8
				15	396.2	15	240.7
				16	392.2	16	214.0
				17	506.8	17	260.0
				18	464.3	18	674.5
				19	481.2	19	657.5
				20	476.3	20	646.3
						21	652.7
						22	826.5

* *The composition of the solutions in each series is identical with those used for Experiments I, II, III and VIII, respectively.*

indicated, it would appear that in normal soil the availability of nitrogen in the nitrate form would really be the most important for the growth of the pathogen.

Discussion

In the work reported, an attempt was made to study, in pure silica sand culture, the incidence of foot rot (*H. sativum*) of the wheat plant in the seedling stage, as affected by four major elements, namely, phosphorus, potassium,

nitrogen and calcium, necessary to plant nutrition. The data do not necessarily apply to the mature stage of the host. Special attention was directed to the effect, on both plant and disease, of the absence, the deficiency, or the excess of minerals indicated.

The disease damage was interpreted according to such pathological manifestations as severity and extent of lesioning, stunting, and outright killing of the plants. Although this method is qualitative, and the one commonly used, quantitative measurements on height and weight of plants proved to be equally satisfactory.

One might naturally expect the wheat plant in an impoverished state, or in an unnatural environment, to be predisposed to attack by *H. sativum*. The data from these experiments strongly support this, since wherever a particular treatment, minus the pathogen, favored the production of undernourished plants, the disease was distinctly accentuated in the parallel series containing the pathogen. This, of course, indicates how closely the lack of host vigor and susceptibility to disease are related.

These studies show that the ions, K, N, and Ca, are necessary in the nutrition of the wheat plant during seedling stage in considerable amounts, if it is to escape excessive damage from the pathogen. On the other hand, the phosphorus ion does not so markedly influence the disease situation, in the seedling stage, for the limits of its effectiveness in maintaining the plant in a vigorous condition could not be measured under the condition of our experiments. Further, optimal concentrations of the K, N, and Ca ions clearly exist. These separate optima vary widely in magnitude. Two general observations seem applicable in the case of all three ions. Decreasing concentrations below the optimum predisposes the plant to more severe disease damage, while the moderate increases above the optimum do not materially affect the plant's response to the parasite. In field fertilizing practice it is possible that the same general principles would apply, although we have not yet attempted to verify this assumption.

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INFLUENCE OF AIR TEMPERATURE AND SOIL MOISTURE SUBSEQUENT TO FLOWERING ON THE NITROGEN CONTENT OF WHEAT[1]

By J. W. Hopkins[2]

Abstract

Marquis wheat plants were grown in soil in the greenhouse under uniform conditions until the flowering stage, when six differential treatments, *viz.*, 15, 20 or 25% soil moisture in combination with a diurnal air temperature cycle of 45–70° or 55–80° F., were imposed.

The effect of the higher air temperature in increasing nitrogen content through accelerated respiration was evident in grain collected when in the early dough stage (about 50% dry matter). By the late dough stage there were also significant differences attributable to soil moisture under both temperature regimes. However, the nitrogen content of the completely ripe grain was practically the same for all six treatments. This is attributed to a retardation of maturity by both increased soil moisture and lower air temperature, which would permit additional dissipation of carbohydrate through prolonged respiration, and also possibly to differences in the extent of tillering.

Compensatory effects of this magnitude would hardly be expected under field conditions, but might occur on a reduced scale, thus increasing the difficulty of correlating nitrogen content with meteorological observations.

In a previously reported (4, 5) statistical study of the influence of weather conditions on the nitrogen content of wheat grown in experimental plots at various stations in western Canada, it was found that above-average precipitation in the earlier part of the growing season was associated with below-average nitrogen content, but that the data did not demonstrate any correlation between nitrogen content and rainfall during the later stages of development. On the other hand air temperature during this latter period was positively correlated with nitrogen content when weighted on the supposition that the rate of respiration was doubled by each 10° C. rise in temperature.

The correlations indicated were, however, only moderate, partly no doubt because of variations introduced by the fact that the plots were not permanent, but were grown on different soil at each station each year. There was thus an appreciable possibility of actually significant effects escaping detection, and it seemed desirable to obtain further data under more closely controlled conditions. The greenhouse experiment described below was therefore undertaken with this end in view.

Experimental

Briefly, the experiment consisted in growing 540 Marquis wheat plants under uniform conditions until the flowering of the main tillers. At this juncture two different levels of air temperature and three levels of soil moisture, making six treatment combinations in all, were imposed upon randomly selected groups of 90 plants and maintained until the conclusion of the experi-

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Published as Paper No. 131 of the Associate Committee on Grain Research of the National Research Council of Canada and the Dominion Department of Agriculture.
[2] *Biologist, National Research Laboratories, Ottawa.*

ment. Collections of the resulting grain, which were made at each of three stages of maturity, were weighed and analyzed for dry matter and nitrogen content.

The crop was grown in 180 numbered 1-gal. glazed earthenware crocks, the tare weights of which were equalized by the addition of varying amounts of coarse (No. 6) quartz sand. Steam-sterilized loam soil (5 kg.) was firmed into each crock, and six grains of Marquis wheat (kindly supplied by the Cereal Division, Central Experimental Farm, Ottawa) planted at a depth of one inch below the surface on October 20, 1936. On November 12 the number of seedlings per crock was reduced to three.

The 180 crocks were arranged in 10 rows of 18 on two benches in a unit of the greenhouse having diurnal temperature control. Each row provided one crock for each of the 18 combinations of treatment and date of harvesting. Since the object was to obtain as accurate as possible a comparison between the six moisture and temperature treatments at each date of harvesting, the 18 crocks in each row were first divided into three groups of six, and one group chosen by random selection for each date of harvesting. Then the six crocks within each such group were allotted at random to the six combinations of temperature and moisture to be subsequently imposed. There were thus ten parallel blocks or groups of crocks, each comprising the six treatments, available for harvesting at each of the three dates.

When weighed into the crocks, the soil contained approximately 20% moisture (oven-dry basis). After seeding, this was brought up to 25% by placing each crock in turn on the scales and adding water until the crock and contents attained the pre-calculated total weight corresponding to that moisture content. By means of regular weekly weighings, the moisture supply of all crocks was then maintained at this level until the date of flowering.

In order to promote uniformity within parallels, the location of the individual crocks within each set of six underwent a cyclical permutation, one move in the permutation being made on the occasion of each weighing and watering. By the repetition of this process positional effects in lighting, temperature and air currents within groups were to some extent equalized.

As the plants increased in size, weekly watering became inadequate, and was supplemented by a mid-weekly supply which was distributed uniformly to all crocks in an amount dependent on the rate of utilization, as indicated by weighing. Actual weighing and movement of the crocks was, however, restricted to weekly intervals.

An initial diurnal temperature cycle of 35–55° F. was adopted, which was progressively raised to 45–70° F. at the time of flowering. When the flowering stage had been passed by the main tillers and attained by the second and third tillers, the 90 crocks allotted to the higher temperature regime were transferred to the adjacent greenhouse unit. The two units now employed were similar in all respects except that diurnal temperature cycles of 55–80° and 45–70° F. were maintained. At the same time three levels of soil moisture

were imposed, the water supply being replenished to 15, 20 and 25% of the oven-dry soil at each weekly weighing, and corresponding modifications being made in the amounts supplied mid-weekly. The principle of arrangement of the crocks on the benches remained the same, each original group of six now being represented by a sub-group of three in each unit. The weekly permutation of crock positions within sub-groups continued as before.

Collections of heads were made at three stages of maturity, *viz.*, early dough, late dough and dead ripe. The first collection was made simultaneously in both units, but as the higher temperature regime perceptibly accelerated senescence, the second was separated by an interval of 12 days in order to secure kernels at approximately the same stage of development. There was a difference of nearly three weeks in the date of attainment of dead ripeness.

It was impossible to thresh out the kernels obtained in the first two collections, which accordingly had to be removed from the heads individually by hand. This was done as expeditiously as possible. After counting and weighing, the kernels from each crock were dried *in vacuo* at 98° C. for approximately 15 hr. in order to destroy enzyme activity which, by the dissipation of carbohydrates, might have led to fictitiously high nitrogen values (6). The dried kernels were subsequently exposed to the atmosphere of the laboratory for three or four days, again weighed, and ground in a micro-mill of the shearing type. Duplicate one-gram samples of the ground material were taken immediately after grinding for the determination of the moisture content of the air-dry grain, which was effected by further drying for one hour in an air oven at 130° C. Finally, the nitrogen content of these samples was determined by the Kjeldahl method, selenium being employed as a catalyst.

Results

Vegetative Features

Table I shows the average number of culms, heads and grain-bearing heads per plant in the 10 replicate crocks of each treatment combination, harvested at the three stages of development indicated above. Under the conditions of

TABLE I

AVERAGE NUMBER OF CULMS AND HEADS PER PLANT AT HARVEST

Collection	Soil moisture	Culms		Heads		Grain-bearing heads	
		45–70° F.	55–80° F.	45–70° F.	55–80° F.	45–70° F.	55–80° F.
First	15%	4.4	4.3	4.1	3.6	3.2	3.3
	20%	7.4	4.2	4.4	4.2	3.1	4.0
	25%	9.0	4.7	4.5	4.5	3.3	3.5
Second	15%	5.1	4.2	4.7	3.8	3.3	3.7
	20%	8.2	4.1	7.3	3.8	3.5	3.2
	25%	10.5	5.1	9.8	4.4	3.7	3.9
Third	15%	5.9	3.9	5.4	3.7	3.8	3.5
	20%	9.0	5.0	8.9	4.4	6.5	3.8
	25%	10.6	5.0	10.4	4.9	8.1	4.1

lower temperature (45–70° F.), there was a considerable proliferation of late tillers at the two higher soil moistures. At the time of the first harvesting, few or none of these had produced heads. By the second harvesting, however, most had headed out, although they were as yet devoid of kernels, and at maturity an appreciable number yielded small quantities of grain. At the higher temperature, on the other hand, this tendency was reduced to very minor proportions.

TABLE II

AVERAGE NUMBER OF KERNELS PER CROCK, FRESH WEIGHT, AND WEIGHT PER THOUSAND

Collection	Soil moisture	Number of kernels		Fresh wt., gm.		Fresh wt. per 1000 kernels, gm.	
		45–70° F.	55–80° F.	45–70° F.	55–80° F.	45–70° F.	55–80° F.
First	15%	267	253	15.2	11.6	56.6	45.1
	20%	276	268	15.6	13.5	55.8	50.5
	25%	274	281	15.4	15.0	56.1	53.3
Second	15%	272	279	14.8	11.6	54.2	41.9
	20%	279	275	15.8	13.0	56.6	47.6
	25%	294	290	16.9	14.1	57.4	49.0
Third	15%	277	277	11.7	9.0	42.3	32.6
	20%	352	288	13.2	9.9	38.0	34.6
	25%	439	296	16.2	10.2	37.4	34.6

The greater vegetative growth under the lower temperature regime is reflected in the average number of kernels per crock of the mature plants, and in the fresh weight of grain secured from both of the last two collections, as shown in Table II. The fresh weight per 1000 kernels of the mature grain suggests that the later tillers produced a preponderance of small or shrivelled kernels, but even so the average is higher than that for the 55–80° temperature regime. Although the latter apparently inhibited late tillering, there was nevertheless a consistent increase in both the fresh weight and weight per 1000 kernels from the 15 to the 25% moisture level at all three collections.

First Collection

When the first collection of heads was taken, the average dry-matter content of the grain from the 30 crocks harvested in the lower temperature unit was 43.4%, whereas the corresponding figure for the higher temperature was 55.7%. Consequently, although the lower temperature gave the greater fresh weight of grain, as indicated in Table III, the actual yield of dry matter at this time was on the average slightly higher in the 55–80° unit, namely 7.4 as against 6.7 gm. per crock. The difference, however, is insignificant.

The variance of the individual yields of dry matter per crock within each unit was analyzed by the well known procedure of Fisher (3) into components ascribable to differences between parallel groups, differences between soil moistures, and residual variation or error. As expected, there were marked positional effects in both units, which gave rise to highly significant mean

TABLE III

AVERAGE DRY-MATTER CONTENT, YIELD AND NITROGEN CONTENT OF GRAIN, FIRST COLLECTION (10 REPLICATES)

—	Diurnal temp. 45–70° F.				Diurnal temp. 55–80° F.			
	Soil moisture			Average	Soil moisture			Average
	15%	20%	25%		15%	20%	25%	
Average dry-matter content, %	44.0	43.3	42.9	43.4	58.5	54.5	54.1	55.7
Average yield of dry matter, gm. per crock	6.7	6.8	6.6	6.7	6.7	7.3	8.1	7.4
Average nitrogen content, % (dry basis)	3.49	3.45	3.41	3.45	3.75	3.78	3.74	3.76

squares between parallels in both analyses of variance. The layout of the experiment was such that average differences between parallels are eliminated from treatment comparisons, which are made within parallels. For the 45–70° temperature regime, the differences in mean yield between the three levels of soil moisture subsequent to flowering shown in Table III are totally insignificant. At the higher temperature, however, there is a significant increase in the yield of dry matter with each increase in soil moisture. The residual mean square error was essentially the same in both cases.

Similar analyses of the variance of the nitrogen content of the grain secured from each crock revealed no significant differences between parallels or between soil moistures at this stage for either temperature. There is, however, as indicated in Table III, an average difference in nitrogen content of 0.31% in favor of the higher temperature regime, which significantly exceeds its standard error of 0.096%. The average nitrogen content, namely 3.45 and 3.76%, is in both cases quite high, corresponding to a protein content on the conventional 13.5% moisture basis of 17.0 and 18.5% respectively.

Second Collection

It has already been mentioned that an interval of 12 days separated the second collection of heads from the higher and lower temperature units. In this way, material at approximately the same stage of maturity was secured, as indicated by the average dry-matter contents of 66.8 and 63.5% respectively shown in Table IV. It is to be observed from this table however that in addition to the effect of temperature in accelerating maturity (as indicated by desiccation) there was also by this time a similar but less pronounced effect within each temperature regime, attributable to the imposed differences in soil moisture supply.

Table IV also shows the yield and nitrogen content of the grain secured from this collection. The yield of dry matter is now somewhat higher than that obtained in the previous collection, particularly in the case of the 45–70° temperature regime, for which the average is 10.1 gm. per crock, as compared with 8.6 gm. for the 55–80° diurnal sequence. The difference,

TABLE IV

AVERAGE DRY-MATTER CONTENT, YIELD AND NITROGEN CONTENT OF GRAIN, SECOND COLLECTION (10 REPLICATES)

—	Diurnal temp. 45–70° F.				Diurnal temp. 55–80° F.			
	Soil moisture			Average	*Soil moisture*			Average
	15%	20%	25%		15%	20%	25%	
Average dry matter content, %	65.4	63.0	62.0	63.5	69.5	66.2	64.8	66.8
Average yield of dry-matter, gm. per crock	9.7	10.0	10.5	10.1	7.8	8.6	9.1	8.6
Average nitrogen content, % (dry basis)	3.77	3.68	3.51	3.65	3.92	3.74	3.79	3.82

1.5 ± 0.37 gm., is statistically quite significant. As before, an analysis of variance showed no significant effect of soil moisture subsequent to flowering upon yield under the lower temperature regime, the late tillers, although well headed out, being as yet devoid of grain. At the higher temperature there is again a consistent increase in mean yield for each increase in soil moisture which, taken in conjunction with the previous results, is suggestive of a real effect, even though the analysis of variance gave in this instance a mean square falling somewhat below the 5% level of significance. The mean square error in the two cases was again of the same order.

At this juncture, the average nitrogen content of the grain developing under the 45–70° temperature cycle was 3.65%, 0.20% higher than that for the first collection, whilst that for the 55–80° temperature, 3.82%, did not differ significantly from the earlier value of 3.76%. The difference between the averages for the two temperature regimes, namely 0.17 ± 0.05% in favor of the higher temperature, is again significant. In addition, there is now a demonstrable effect of soil moisture under both temperature conditions, higher soil moisture being associated with lower nitrogen, except in the case of 25% soil moisture at 55–80° F., which does not differ significantly from the 20% series.

The possibility of correlation between grain yield and nitrogen content was investigated by partitioning the total covariance of these quantities within each temperature regime in the manner already employed in the analyses of variance, *i.e.*, between parallel groups, between soil moistures, and residual. In both cases the residual covariance gave rise to a small negative correlation coefficient, but neither of these attained the 5% level of significance.

Final Collection

The plants under the higher temperature cycle matured more rapidly, and hence were actually harvested prior to those in the adjoining unit. As indicated in Table V, the average dry-matter content of the grain at the time of collection, namely 90.3 and 89.4%, was much the same in both cases.

Table V also shows the average yield of grain, expressed as grams of dry matter per crock, and the average nitrogen content of each series. The average yield for the 10 replicate crocks of the 45–70° series, 12.3 gm., is significantly higher than the corresponding figure of 8.9 gm. for the 55–80° series. There is now a significant effect of soil moisture upon yield in both series, but this is much more pronounced at the lower temperature owing to the fact that many of the late tillers, when allowed to mature, produced small quantities of grain. Positional effects, reflected in the differences between the average yields of the 10 replicate groups of each series, were again highly significant in the analyses of variance, as in the two previous collections. The residual mean square error was somewhat higher in the 45–70° than in the 55–80° series, the yield of grain from the additional tillers of the former doubtless introducing an extra source of variability.

TABLE V

AVERAGE DRY-MATTER CONTENT, YIELD AND NITROGEN CONTENT OF GRAIN, FINAL COLLECTION (10 REPLICATES)

—	Diurnal temp. 45–70° F.				Diurnal temp. 55–80° F.			
	Soil moisture			Average	Soil moisture			Average
	15%	20%	25%		15%	20%	25%	
Average dry-matter content, %	89.7	89.3	89.1	89.4	90.4	90.3	90.3	90.3
Average yield of dry matter, gm. per crock	10.5	11.8	14.5	12.3	8.2	9.0	9.3	8.8
Average nitrogen content, % (dry basis)	3.83	3.94	3.81	3.86	3.84	3.83	3.92	3.86

Turning now to the nitrogen content of the ripe grain, given in the bottom line of Table V, it will be observed that the average for both temperature regimes is now the same at 3.86%. Furthermore, analyses of variance indicated that the small differences between the averages for the three soil moisture levels were in both cases insignificant, although the pooled residual covariance of yield and nitrogen content gave rise to a small but probably significant correlation coefficient of −0.38, and, as noted in the preceding paragraph, there are undoubted differences in yield attributable to both moisture and temperature conditions. This final uniformity of nitrogen content is believed to be due to the fortuitous balancing of opposed effects, since definite differences were discernible in the developing grain.

In this connection, three points may be noted. (i) The grain under the 55–80° temperature regime was fully ripe on the average between two and three weeks earlier than that under the 45–70° regime. (ii) Soil moisture was maintained at the predetermined level of 15, 20 or 25% right up to the time of the final harvesting. This resulted in differences in date of maturity at both temperatures, the plants receiving the smaller quantities of moisture maturing more rapidly. It may be assumed from the results of the second

collection of immature kernels, summarized in Table IV, that the additional vegetative growth resulting from increased soil moisture led to some dilution of the nitrogen content of the parent material translocated to the grain. On the other hand, however, the additional moisture supply operated to retard desiccation, thus permitting a prolongation of active respiration, which in the end apparently sufficed to offset the former effect. Similarly, the results in both Tables III and IV indicate that the higher diurnal temperature cycle caused an increased dissipation of carbohydrate through respiration. Here again, however, desiccation supervened earlier, and the lower rate but more prolonged duration of respiration at 45–70° apparently contributed to an equivalent result. (iii) The final production of grain by a number of the late tillers generated under this temperature regime may also have operated in the same direction, since Engledow and Wadham (2) found the percentage of nitrogen in the grain of mature ears of barley to increase in sequence from the main axis down through the successive side tillers.

Since the experimental conditions were in certain respects artificial, the foregoing compensatory effects are doubtless exaggerated in comparison with those occurring under field conditions in western Canada. Apart from the tillering differences noted, the maintenance of a continuous supply of soil moisture during maturation is a situation hardly likely to be encountered in practice. Also, it has long been known (1, p. 68) that the respiration of greenhouse plants is abnormally high. However, the presence of such effects, even in moderate degree, may contribute to the difficulty encountered in correlating nitrogen content with the meteorological conditions prevailing during the formation and ripening of grain crops.

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NOTE ON SULPHANILAMIDE AND OTHER CHEMICALS THAT ACT AS PLANT GROWTH PROMOTING SUBSTANCES[1]

BY N. H. GRACE[2]

The response of both higher and lower plant forms to growth stimulating chemicals suggested a possible relationship between the action of the so-called phytohormones and that of other chemicals or drugs used in controlling bacterial growth. A study of several plant extracts indicated the presence of active material not to be accounted for by heteroauxin or auxin as such. The chemical separation of the active materials was started by Dr. W. E. Graham of these laboratories: his work was cut short by his untimely death, and in consequence, a detailed report on the project has been delayed. However, his work suggested testing a number of chemicals for their physiological activity as growth promoting substances, including some of natural plant origin and others which are products of artificial synthesis, such as sulphanilamide.

The yeast method of testing was used as an indicator of activity (3). Following this, the chemicals were applied to cuttings of easily rooted plants. The response was compared with that obtained with indolyl-3-acetic acid. The physiological curve, already reported as characteristic of the effects of hormones, was demonstrated for these additional substances by application to tomatoes grown in sand culture. In some cases a response was noted with seeds.

Another method of determining the activity of the recognized plant hormones and new chemicals is being tested independently by Dr. N. E. Gibbons and Dr. G. A. Ledingham of these laboratories, who will report their experiments in due course. They verified inhibitory concentrations of substances mentioned herein by the clear zone of inhibition occurring around a pellet of talc containing the active chemical, applied to the surface of Petri dish cultures of bacteria or fungi.

The foregoing methods indicated that coumarin, vanillic acid, 1- and 2-γ-naphthyl butyric acid (3) and sulphanilamide had a definite measure of activity as growth promoting substances. The response was comparable to that shown by both indolyl-3-acetic and 1-naphthyl acetic acids, though it was not as great in all cases. In lesser measure, piperonal, methoxy-salicylaldehyde and vanillin also indicate activity. Observations with yeast alone indicate some activity for iso-vanillin, piperonal acetic acid and colchicine.

Perhaps the most interesting results are those obtained with sulphanilamide. It gives a response with yeast and stimulates the proliferation of roots by certain plants that root readily. A physiological curve of stimulation and inhibition follows its application to tomatoes in sand culture. A clear-cut

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa.
[2] *Chemist, National Research Laboratories, Ottawa.*

response follows its addition to seeds. In all these cases sulphanilamide acts like the recognized plant hormone chemicals. It would appear to follow that sulphanilamide does possess some growth promoting properties.

This similarity in the behavior of plant hormones and sulphanilamide suggests that the therapeutic effectiveness of the latter may be attributed, in some measure at least, to its hormone-like properties. In other words, the effect on bacterial infections may be due to overdosage. The concentrations employed therapeutically are such that inhibition of growth results; inhibition similar in nature to that shown by higher plants, fungi or bacteria, when they are treated with excessive doses of indolyl-3-acetic or 1-naphthyl acetic acids.

Reference to the current literature on sulphanilamide and the group of related chemicals indicates no clear concept of the mechanisms involved (1, 4). The analogy with phytohormones would suggest looking for a common cause and mode of action. Two conclusions would appear to follow. In the first place, certain low concentrations of sulphanilamide might be expected to stimulate, rather than control, the infection. With phytohormones it is usually much easier to show inhibition than stimulation. Moreover, a number of factors are involved in stimulation and it may be difficult to show it under the complex conditions which exist for growth of organisms in the animal body. In the second place, phytohormones should be considered as a possible means of checking the growth of undesirable micro-organisms.

In conclusion, it may be pointed out that other apparently active chemicals are under study in this laboratory. Various workers report numerous materials affecting plant growth (2). Where may the line be drawn between a true phytohormone and a material exhibiting toxic effects at high concentrations and stimulating effects at low? The number of active materials now known suggests some such division is desirable.

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MICROBIOLOGICAL STUDIES OF APPALACHIAN PODSOL SOILS

III. SYNCHRONOUS CHANGES OF BACTERIAL NUMBERS IN TWO FIELD SOILS[1]

BY P. H. H. GRAY[2]

Abstract

Determinations of the numbers of bacteria (and actinomyces) in two cultivated podsol soils, sampled on the same days at intervals of one month during the growing season of 1937, showed that numbers developing in plates of two agar mediums fluctuated extensively and in the same manner in both soils. The fluctuations were of greater amplitude in plots treated several months previously with limestone, with sodium carbonate, and with both amendments together, than in the control plots. Numbers in the late spring (June 14) were from two to four times the numbers found in the summer (August 11). In the spring the less selective medium (soil-extract–dextrose agar) gave higher numbers than the more selective medium (Thornton's mannitol-asparagine), but in the summer the numbers were the same in the two mediums.

Introduction

The main purpose of estimating the density of the soil microflora has been to find some relation between numbers of organisms and the factors affecting crop yield. This method of approach has not hitherto proved to be sound, since the plating method restricts the number or kinds of micro-organisms entering into the estimate, and since seasonal changes in numbers have proved to be of such amplitude that numbers found at one time cannot be accepted as representing the actual state of the flora at all other times during the growth of the crop.

The plating method does, however, give evidence of the effects of some treatments, such as liming, that are expected to affect crop yields. Liming appears to encourage the activity of the nitrifying bacteria, but there appears to be no evidence that lime added to field soil stimulates the decomposition of organic compounds of nitrogen and the production of ammonia therefrom, thus furnishing the nitrifying bacteria with increased supplies of oxidizable nitrogen, although the numbers of aerobic heterotrophic bacteria usually associated with such decompositions may be increased by liming (1, 6). Other soil amendments, such as calcium oxide and sodium carbonate, which release organic matter in acid soils (7), have also been shown to stimulate nitrification as well as the multiplication of the heterotrophic bacteria in field soils (1).

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Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que., Canada. Macdonald College Journal Series No. 93. This paper is the third in a series dealing with part of the work of the Soil Fertility Committee of Macdonald College.

[2] *Associate Professor of Bacteriology, and Head of the Department of Bacteriology, Macdonald College.*

Jensen (5) has recently shown that the numbers of bacteria, actinomycetes and fungi in 50 cultivated soils of New South Wales, Australia, were separately correlated with the organic matter. The relationship was also shown to exist in the separate horizons of heavily podsolized soils of Quebec (2, 3). Such relationships as have been reported, however, have been based on that between the micro-organisms and the total organic matter, as determined by *ignition*; a better understanding of the relation between the heterotrophic soil flora and the organic matter may be attained only after analysis of the material in the soil solution. It is also necessary that such a relation be established after knowledge has been attained as to the magnitude of the fluctuations that take place seasonally among the organisms.

Seasonal fluctuations of microbial activity in Appalachian podsol soils have been reported (2), as occurring synchronously in soils in areas as much as 25 miles apart. In that work, the samples of soil were collected at any one place 14 days after collecting samples at the place previously visited. The results, though clearly pointing to the existence of fluctuations of the same order and in the same direction, in soils so widely separated, remain open to criticism in that there was no evidence that fluctuations of some extent did not occur during the six-week intervals between the times of sampling at any one place.

In attempting to show the existence of an association between the density of the microflora and available organic matter, two lines of approach have been chosen; first, to determine the density of micro-organisms in soils receiving such amendments as are known (6) to release organic matter; and, secondly, to add easily decomposable compounds of carbon and nitrogen to soil and then to determine the subsequent biological activity. The studies reported below were concerned with the former of these lines of approach.

Experimental

The present work was undertaken to ascertain whether fluctuations occurring at one place would be the same, either in degree or direction, as those occurring at another place where samples were taken on the same day. In order to obtain samples of a uniform nature, advantage was taken of plots laid out in October 1935, at two farms near Sawyerville in the Eastern Townships of the province of Quebec, for the purpose of studying the effects of limestone and sodium carbonate, alone or in combination, upon the soil and crop yields. The soils were situated a few miles apart, and were of similar origin; they differed in that one had only recently been brought into cultivation.

The plots at each place were 16 in number, each 1/200 acre in area, and were arranged in 4 rows, one plot in each row being kept without treatment. The plots were sown with oats, seeded to clover and timothy grass, in the spring of 1936. Samples for the work reported here were taken first in 1937 on the dates shown in the tables. Five cores of soil, 6 in. deep, were taken from each of the four plots and thoroughly mixed; from the composite samples of 20

cores, a sample of about 4–5 lb. was packed in a can for determinations in the laboratory; the sample usually reached the laboratory within 48 hr. of sampling. In the laboratory the whole composite sample was passed through a 3-mm. sieve, and determinations were made for moisture in fresh soil, pH, and numbers of bacteria and actinomyces developing in two agar mediums.

Since the values for moisture in fresh samples were used merely to assist in estimating results on a basis of dry soil, these values are not quoted. Also, since the present paper does not aim to consider the effects of treatment on biological activity, the pH values of the samples are not reported. It may be mentioned, however, that the moisture content of the soil, *T*, recently brought under cultivation, was consistently higher than that of the other soil, *R*, and that the treatments with ground limestone, or sodium carbonate, or both, raised the pH values from about 5.0 to 6.5. Such fluctuations of the values of these two factors as were found could not be associated with the fluctuations in microbial activity.

The mediums used were Thornton's mannitol-asparagine medium and soil-extract–dextrose agar. The former medium was selected for its known value in detecting significant fluctuations in numbers of bacteria in soils of varied types; soil-extract–dextrose agar was selected for the reason that, being less selective, it might prove useful in determining the density of larger groups of bacteria especially associated with the decomposition of organic compounds.

TABLE I

BACTERIA AND ACTINOMYCES, MILLIONS PER GM., THORNTON'S MEDIUM

Plots	May 13	June 4	July 13	Aug. 11	Sept. 16
			Soil R		
Control	6.02	10.15	5.27	5.84	7.37
Limestone	14.40	16.94	9.17	6.55	13.14
Sodium carbonate	8.76†	19.85†	11.76	4.19	22.59
Both amendments	14.63	27.21	15.57	6.30	9.42
			Soil T		
Control	10.85	12.40	8.89†	8.77	21.45
Limestone	31.39	48.25	30.31	14.79	16.96
Sodium carbonate	26.99	40.63	20.65	16.05†	8.81
Both amendments	36.64	40.84	21.92†	13.13	17.73†

† χ^2 *excessive;* $P < 0.02$.

The soil extract was prepared by autoclaving 1 kg. of soil with 1000 ml. of distilled water, filtering hot through filter paper, and making the filtrate up to 800 ml. To this extract, K_2HPO_4, 0.02%, and agar 1.5% were added. Before sterilizing, dextrose, 0.5%, was added; the reaction was not altered. Each medium was usually sterilized in measured 50 ml. quantities in Erlenmeyer flasks of 125 ml. capacity, and melted just before use.

The numbers of bacteria and actinomyces, estimated by counting colonies developing in five plates after ten days at 25° C., are given in Tables I and II.

TABLE II

BACTERIA AND ACTINOMYCES, MILLIONS PER GM. SOIL EXTRACT MEDIUM

Plots	May 13	June 14	July 13	Aug. 11	Sept. 16
			Soil R		
Control	26.8	15.6	5.7	6.5	15.2
Limestone	(67.0)*	28.6	9.1	9.6	19.9††
Sodium carbonate	38.4	48.8	11.8	5.8	30.6††
Both amendments	(82.0)*	Lost	19.5	6.7	19.0
			Soil T		
Control	21.0	26.4	18.1	10.5	44.9
Limestone	48.3	56.1	33.4	12.9	28.0††
Sodium carbonate	(48.3)†	59.3	16.4	13.4	22.5
Both amendments	(60.3)†	50.4	24.0	11.8	28.8††

* *More than 500 colonies.* † *From single plates. These values were omitted in calculating the average numbers in the treated plots, as shown in Fig. 2.*
†† χ^2 *excessive;* $P < 0.02$.

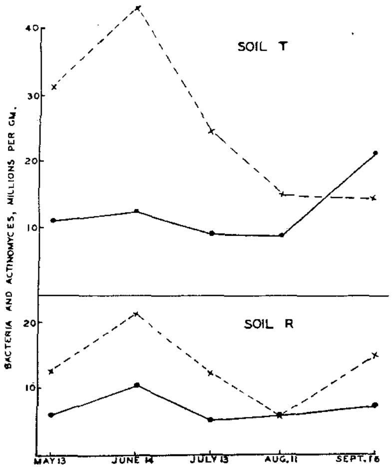

FIG. 1. *Changes of bacterial numbers in 1937, in Soils R and T; Thornton's medium. Solid line, control plots; broken line, treated plots.*

The values estimated from the number of colonies found in only one plate of a series (the remainder were spoiled by fungi or spreading colonies) and those estimated from the mean numbers of colonies in plates, the χ^2 of which was excessive, are noted in the tables.

The results for numbers of bacteria and actinomyces are shown also by means of graphs in Figs. 1–3.

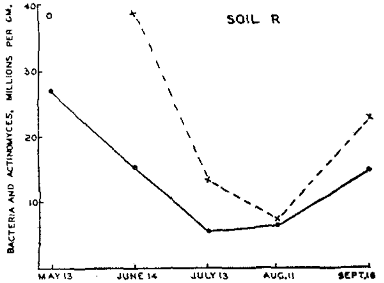

FIG. 2. *Changes of bacterial numbers in 1937, in Soil R; soil-extract agar. Circle, sodium carbonate plots only. Solid line, control plots; broken line, treated plots.*

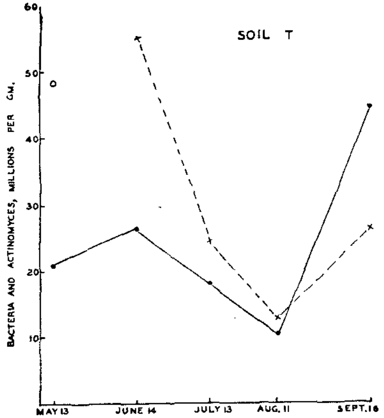

FIG. 3. *Changes of bacterial numbers in 1937, in Soil T; soil-extract agar. Circle, limestone plots only. Solid line, control plots; broken line, treated plots.*

Conclusions

The two soils differed in biological activity, in that the mean level of numbers of bacteria and actinomyces was higher in soil T, which had been brought into cultivation only a few months preceding the year of sampling. The mean levels of numbers are shown in Table III.

TABLE III
BACTERIA AND ACTINOMYCES, MILLIONS PER GM.

——	Thornton's medium	Soil extract medium
Soil T	10.2	19.0
Soil R	6.8	13.7

The values are the average of the numbers of organisms in Samplings I to IV from the plot that had not been treated; the values from Sampling V are omitted, since there was an unavoidable interval of five days between collecting and plating.

There appears to have been a common factor affecting the numbers of micro-organisms throughout the season at the two places. Considering the more selective medium first, there was a rise in numbers in the middle of June, followed by a fall, which was maintained for two months. There appears to have been a tendency for numbers to increase in September, the average of the numbers in Soil R in that month being more than twice those in August. The fluctuations were greater in the treated plots, and of relatively greater amplitude in Soil R than in Soil T, though numbers in the latter soil were consistently the higher.

Unfortunately, some of the values obtained by plating with the less selective soil-extract medium cannot be accepted as reliable. In spite of these, however, two points of interest seem to call for notice: first, the higher numbers developing at first in this medium as compared with the more selective medium were not maintained throughout the season, notably in August, when they were about the same in the two mediums; and, secondly, in September the numbers were twice or three times as great as in August.

The results obtained by the use of the less selective medium are of further interest in that the numbers in the treated plots in the August sample were at the same level as those in the control plots.

The ratios between the numbers in the June samplings and the August samplings are found to be 4 : 1 in the soil extract agar, and 3 : 1 in the mannitol-asparagine medium. It would appear from this that groups of bacteria that were active in the spring in both soils were removed by factors common to both soils. Some of the bacteria were evidently those stimulated by the limestone and sodium carbonate, the effects of which were operative mainly in the spring.

It is possible to compare these results with those reported previously for one soil situated in the same locality (Soil S in Reference (2)). The changes in 1931 were unlike those of 1937, only in that in the earlier year higher numbers were found in May than in June; in 1932, the higher numbers occurred

in August; the fluctuations in 1933 resembled those of 1937 in direction but not in degree.

In spite, therefore, of the agreement found in the nature of bacterial activity in two widely separated soils, it does not seem possible to accept the changes found to occur in any one year as part of a normal recurring cycle, which can be ascribed to external climatic influences in these soils.

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

I. GENERAL INTRODUCTION[1]

By A. G. Lochhead[2] and C. B. Taylor[3]

Abstract

Soil microbiological research has been directed for the most part towards a study of processes in which micro-organisms are known to participate rather than towards an objective study of soil micro-organisms themselves. While organisms concerned with known processes have been given much study, relatively little attention has been paid to groups of bacteria whose functions are as yet unknown or but little understood, but which are believed to comprise a very large proportion of the micro-population of arable soils. A review is made of investigations based on the biological, as contrasted with the biochemical (or functional) approach to soil microbiology. Qualitative studies of the general soil microflora are regarded as essential to a better understanding of microbiological activity in soil and its relation to practical problems of crop growth, soil borne plant diseases, and general soil fertility.

Approach to Soil Microbiology

Soil microbiological research in the main has been directed towards studying microbiological processes rather than the micro-organisms themselves. The rise of bacteriology in the latter half of the nineteenth century led to an immediate and phenomenal application to medicine which gave hopes of an equally effective application of the new science to problems of soil fertility. Coincident with the discoveries, in the closing decades of the century, of the role of bacteria in human and animal disease, equally brilliant if less spectacular discoveries were made of the part played by micro-organisms in many soil processes.

Right up to the present the study of processes, and incidentally that of the specialized groups of organisms concerned in these processes, has occupied by far the greater part of the attention of most soil microbiologists. Valuable data have been gathered on the numerous biological processes known to occur in soils, such as ammonification, nitrification, nitrogen-fixation, processes concerned with the transformation of sulphur and other elements, the decomposition of plant residues and miscellaneous organic compounds. Detailed investigations have been made of bacteria and other micro-organisms known to take part in such processes. However, such organisms have been studied, not so much from an interest in them as organisms, but because of their known, and presumably important functions.

The immediate application of bacteriology to medicine, and a similar concentration, in the case of soil microbiology, on functions, has if anything delayed progress in the objective study of bacteria. Even today, when microbiology is so widespread in its application, many of the fundamental questions of the morphology and physiology of bacteria remain unanswered or at least in active dispute.

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Contribution No. 47 (Journal Series) from the Division of Bacteriology, Dominion Experimental Farms, Ottawa.
[2] *Dominion Agricultural Bacteriologist.*
[3] *Graduate Assistant.*

In soil, bacteria are indigenous to the medium in a sense not true of bacteria in milk, in foodstuffs or in blood or living tissues. In arable soil we have a centuries-old equilibrium, though admittedly an ever-changing one, not analogous to conditions prevailing, *e.g.*, in a given sample of milk or in an infected animal. In the latter instances, too, we can better recognize cause and effect; we have to deal with fewer antagonisms and associations of different groups of organisms; and we have a better conception of the functions of the organisms in the medium. In the soil we can perceive many biological processes. Some of these we can evaluate fairly well; others, such as non-symbiotic nitrogen-fixation, we are unable to evaluate though the organisms able to exercise the function under artificial conditions have been given much study. There is reason to believe, however, that the organisms in soil which take part in recognized soil functions are greatly outnumbered by those whose functions are yet unknown.

Greater cultural difficulties doubtless stand in the way of a biological, as contrasted with a biochemical (or functional) approach to soil microbiology. We require a non-selective rather than a selective medium to permit of the isolation and study of the greatest numbers of types occurring in soil. Much more success has been achieved in the development of highly selective culture media than in the development of non-selective media, and of the latter type the most we can say is that they are less selective than the others.

Our least selective media are used for the most part for "total counts" of soil organisms. The enumeration of plate colonies constitutes the only consideration usually given to many types of micro-organisms characteristic of soils and forming a large part of their total population. The importance of many types in soil is likely to be gauged by the size of their respective colonies, very many of which are small or of pin-point size. This impression may be strengthened by casual microscopic and physiological tests which indicate, in a large number of cases, small, rod-shaped organisms, relatively inactive as judged by our standard artificial testing procedures. At the present state of our knowledge, however, micro-organisms that do not appear to fit into our more important physiological groups as we recognize them today, cannot be dismissed as unimportant. More study of an objective nature is required before we can reliably assess such groups. Their undoubted abundance in most soils renders essential a more thorough knowledge of them than we possess at present; such knowledge would help to form the basis of a more complete understanding of soil micro-organisms in general.

The General Bacterial Microflora of Soil*

Our present knowledge of the general microflora of the soil, as distinct from types of micro-organisms concerned with known biochemical processes, is due to a comparatively small number of investigators, and in a large measure

* *This paper is concerned primarily with bacteria, the most abundant soil organisms and the group studied relatively least from the purely qualitative point of view. It is recognized that actinomyces, fungi, algae and protozoa comprise important divisions of the micro-population of soil.*

to the work of Conn, who extended and developed ideas underlying the earlier work of Chester. Previous investigators, to be sure, had reported studies of bacteria isolated from soil. As early as 1881, R. Koch (26) made the observation, which has been repeatedly confirmed since, that rod forms are greatly in excess of cocci. As was but natural from the prominence of their colonies on beef gelatine or agar plates largely employed in earlier work, spore-formers received particular attention. Houston (21) describes various types of this group, devoting less attention to non-spore-forming organisms. He and other contemporaries of Chester, such as Gottheil (19) and Neide (31) who studied spore-formers, were apparently more interested in definite groups than in the whole microflora of soils.

In 1900 Chester (1) first emphasized the importance of a knowledge of the types of organisms predominating in soil. Introducing a study on bacterial classification he enunciated a principle which has received all too scant attention.

"Agricultural bacteriology is destined to have a very important bearing, but as yet is without any foundation. The animal pathologist deals with a comparatively few forms which he can readily identify. The agricultural bacteriologist, on the other hand, can scarcely take up a piece of work before he meets with scores of bacterial forms of which he knows nothing, and which he is unable to identify. Hence the first desideratum before he can advance in this important field is to possess some system of bacterial classification, however crude and imperfect. These studies in bacterial classification have been preliminary to the investigation of the bacterial flora of cultivated soils. Inasmuch as soil bacteria are the active agents for the digestion and elaboration of plant food in soil it is important to know something about them, not only collectively but individually. It is necessary to know what species of bacteria are commonly present in all soils and the part each plays in plant food elaboration."

In the same year Chester (2) described miscellaneous types of bacteria isolated from soil, and in 1903 (3) published what is probably the first study of the predominating bacteria of soil. From gelatine plates of 1/100,000 dilution of soil, giving but small numbers of colonies, the predominating types of organisms were studied in detail. The three main types in order of abundance were named, following Migula's system of classification, *Streptothrix soli*, *Bacterium flocosum* (a non-motile spore-former) and *Bacillus Delavariensis* (a motile non-spore-former). This and related studies by Chester (4), such as a special investigation of spore-forming bacteria in soil, represent the most important work up to that time on the qualitative nature of the soil microflora. It was pointed out by the same author (5) that in order to form a true estimate of what is taking place in soil through the agency of bacteria we should understand the function of the different types. He stressed the importance of isolating all types which predominate in soil and of a quanti-qualitative soil microbiological analysis.

Chester was breaking new ground while bacteriological methods were still far from perfect. The principles involved in his work are perhaps more

important than the findings obtained, and deserve more consideration than has been accorded up to the present.

In 1903 Hiltner and Störmer (20) made a study of types of bacteria in soil, on lines of general groups rather than of definite species. Three main groups were recognized, liquefiers, non-liquefiers and Streptothrix. The numerical importance of spore-formers in soil was put in doubt by studies which showed that they comprised but a small percentage of colonies on plates whereas non-liquefying, non-spore-forming organisms formed by far the largest group. Whereas Chester (5) inclined to the belief that the prevalence of kinds of bacteria in a soil was largely a fortuitous matter, with relatively few species predominating, Hiltner and Störmer found the relative numbers of the broad groups to be fairly constant in normal soils, suggestive of a certain state of equilibrium.

In a series of studies first appearing in 1917, Conn (7, 8, 9, 10, 12) added much to our qualitative knowledge of soil bacteria through extensive work on the general soil flora as contrasted with the more intensive work on special groups of organisms that were considered important on account of their physiological activities and commanded most attention from contemporary soil biologists. Conn's provisional classification recognized five main groups in soil as determined by studies of colonies on gelatine and agar plates:—(i) spore-formers, (ii) rapidly liquefying, non-spore-forming short rods, (iii) slowly liquefying or non-liquefying, non-spore-forming short rods, (iv) micrococci and (v) Actinomyces. Of these, Groups (ii), (iii) and (v) were the most numerous, the slowly liquefying or non-liquefying short rods being the most abundant and doubtless comprising the same broad group recognized by Hiltner and Störmer. Martin (30) likewise found non-spore-formers to comprise the majority of organisms in normal soil with Actinomyces next in abundance, and spore-formers occurring in smaller numbers. In a study of Texas soils, however, Williams (38) reported having found spore-forming bacteria as the dominant types from an examination of colonies isolated. However, as no attempt was made to determine the relative abundance of different forms occurring on plates it is not possible to assess the relative incidence of the various types.

In studying the predominant types occurring in frozen soil Lochhead (29) showed the largest group to consist of slowly liquefying or non-liquefying, non-chromogenic short rods, which group represented in even more pronounced degree the majority of bacteria capable of growth at low temperature (3° C.). Actinomyces, though unable to grow at low temperature, comprised the second largest group. Non-spore-forming, liquefying short rods, spore-formers and micrococci formed numerically much less important groups. The relative abundance of the different groups in frozen soil corresponded closely with the findings of Conn and led to the belief that, as far as the main types are concerned, the winter flora of soils differs little from the summer flora.

The rapidly liquefying short rods, Group (ii) of Conn's classification, were apparently closely related to *Pseudomonas fluorescens*. Though forming a

relatively small portion of the total microflora, they were found by Conn to be specially abundant in freshly manured soil, a finding confirmed by the work of Lewis (28), and with their distinctly proteolytic properties were suggested as being important soil ammonifiers.

The great majority of the non-spore-forming organisms, made up largely of the group of slowly liquefying or non-liquefying short rods, were less active physiologically and grew less abundantly on the media used. Organisms of this group were referred to at first as "slow growers" by Conn, and later as "punctiform colony forming bacteria" on account of the restricted size of colonies on tap water gelatine. While several sub-divisions of this group were made (14), special attention was given to two types which embraced the great majority of the forms studied:

(I) Small, short rods, motile or non-motile, with no tendency to produce irregular forms. With this sub-group many variations in staining properties and physiology may be observed. This suggests either the existence of many species within the sub-group or unstable physiological characters.

(II) Pleomorphic forms, appearing as short rods in young culture but changing into cocci within a few days. While variability in staining and physiology occurs, it is less pronounced than in the previous sub-group.

In addition to the above, other much less abundant sub-groups (III and IV) were noted, less definitely classifiable, but showing a tendency to produce filaments, branched or unbranched. These forms are doubtless related to soil organisms showing branching and described as members of the genera *Corynebacterium*, *Mycobacterium* and *Proactinomyces* by Jensen (22). It is probable that such organisms with tendency to produce branched forms may comprise relatively large proportions of the micro-population in some soils. Thus in Australian soils Jensen (23) found corynebacteria to comprise from 8 to 65% of the colonies appearing on dextrose agar plates. *Mycobacteria*, however, were found by Jensen (24) to occur much less frequently, though Krassilnikow (27) states that they are widely distributed in certain Russian soils, especially those rich in humic substances.

Organisms of the sub-group (II) above, comprising the cocci-forming rods, gave evidence of a much closer inter-relationship than those of (I) and were regarded as consisting almost wholly of one species, to which the name of *Bacterium globiforme* was given. To this organism, one of the predominating types in the soils studied, special attention has been given by Conn (13, 14) and Conn and Darrow (16, 17), particularly in view of its apparently greater abundance in many productive soils than in certain less productive soils investigated.

Even in soils that may be classed as abnormal the main groups recognized by Conn appear to be present, though under extreme conditions such as are represented by arid or desert soils the proportions may be considerably altered. In a series of investigations summarized by Snow (33) studies were made of the bacterial flora of wind-blown soils from six localities. In but one of the soils studied was the average total count in excess of 100,000 per gram.

Examination of the types isolated suggested that cocci, liquefying short rods, and in most cases spore-forming rods, formed relatively larger proportions of the cultures from such soils than of those from more arable soils.

Coincident with progress in soil flora studies from the cultural side has been a development of methods for the direct microscopic study of soil organisms. The first method, proposed by Conn (11), was essentially an adaptation of the Breed smear method for milk examination, and consisted in the staining of a suspension of soil after fixing and drying on a slide. This procedure, with some modification, was later used by Winogradsky (40, 41) in connection with his "direct method" of soil study. A very important development was made by Rossi and Riccardo (32) who first advocated the use of the direct contact slide method and provided a new means of studying not only the forms, but also colonies of micro-organisms, as they occur in soil, and other points of interest not brought out by the stained suspension method. A very important modification of this, the most direct method, was made by Cholodny (6), while further adaptations were suggested by Conn (15) and others for the examination of soil *in situ* or in the laboratory, with any desired modification or treatment.

By the aid of the microscopic method, Winogradsky (39, 41) was able to study the main morphologic types in soil and particularly their reaction to changes in environment, such as are caused by the addition of nutrient materials. He classified soil organisms in two main categories. One consists of the *autochthonous* (*i.e.*, indigenous) flora, characteristic of soils poorly supplied with fermentable substances. Organisms of this group are for the most part oval forms or cocci, comparatively inactive and believed to take part in the slow combustion of the humic constituents of soil. Another category was recognized which he calls the *zymogenic* organisms. These are scarce in normal soils but become very active upon addition of any readily fermentable substance. In this group are included the spore-formers, which as Joffe and Conn (25) had shown, are apparently inactive under ordinary field conditions but may multiply upon addition of fresh available organic matter, particularly when abundant moisture is present, and engage in decomposition processes.

Apparently Conn's group of non-spore-forming bacteria corresponds essentially to Winogradsky's autochthonous group, representing the indigenous soil organisms as contrasted with other types which come into prominence mainly under special conditions. The agreement at first was not so evident. Winogradsky assumed that the autochthonous group was largely incapable of being cultivated on ordinary media from the fact that, whereas cocci forms predominated in the microscopic examination of soil, relatively few cocci developed in cultures. However, Thatcher and Conn (35) found that in some soils as many as 40% of the organisms growing on plates may consist of coccus-forming rods. This work was followed by the studies of Conn, and Conn and Darrow, referred to above, and by the recognition of the *Bacterium globiforme* group as an important part of the autochthonous soil flora.

Further application of the microscopic method has been made by various workers in studying the relative abundance of different groups in soil, particularly as affected by soil treatment. Thus the work of Demeter and Mossel (18) and of Vandecaveye and Villanueva (37), though carried out by different modifications, showed that useful application could be made in indicating qualitative changes on a broad basis with approximative quantitative values. The method, however, is inadequate for studies of the role of the organisms in soil. The soil slide method provides us, to be sure, with an additional and valuable means for soil flora investigation. It possesses certain advantages and also the limitations of microscopic methods. From a qualitative point of view it may be used to advantage in noting the prevalence of different morphologic types and their reaction to environmental changes. It is therefore a useful supplement to cultural studies, but the latter, however, are necessary for an adequate study of the unknown organisms in soil and their possible functions.

Of the autochthonous microflora it would appear that *Bacterium globiforme* (or the *Bact. globiforme* group) comprises a significant part, though relatively little attention has been accorded it. Conn (13, 14, 16, 17) studied the physiological properties of the organism and furthermore noted certain relationships between its incidence and soil productivity. Of interest was its occurrence in certain fertile soils and its absence from certain less productive soils, suggesting that the inability of the latter to support growth of *Bact. globiforme* was associated with their relatively low crop-producing ability. The work of Conn and Darrow (16) suggested further that the growth of the organism in soil was dependent upon the presence of readily available nitrogen, which is lacking in the poor soils. Further work by the same authors (17) led to the conclusion that the organism retains, in the soil, nitrogen that has been converted by other organisms into a soluble form and which otherwise would be removed by drainage or utilized by plants. Depending upon conditions, therefore, the organism may be beneficial or harmful, with the beneficial function predominating.

In comparing the incidence of *Bact. globiforme* in soils differing in fertility, Taylor and Lochhead (34) found, by quanti-qualitative methods, no indication of relationship between the abundance of the organism and the productivity of the soils in question. There was more indication of an influence of the crop on the bacterium, though in all cases it formed a significant part of the total microflora. The findings obtained, when compared with those of Conn and Darrow, suggest that inability of certain soils to support growth of *Bact. globiforme* may be related to some special factor affecting productivity and not to general lack of crop-producing power.

The most recent study of the predominant micro-organisms in soils is that of Topping (36), who made an examination of the organisms which occurred most numerously in a series of soils from southeastern Scotland and Saxony. Gram-positive, non-spore-forming, non-acid-fast rod forms were found to outnumber all other types, as determined by a study of organisms growing

from the highest dilutions of soil on a variety of media. These Gram-positive bacteria were divided into three groups: (1) motile organisms producing branching variants, (2a) non-motile non-branching rods and (2b) non-motile, mycelium-forming types. All three groups represented forms exhibiting considerable pleomorphism. Organisms of Groups 1 and 2a particularly showed that the production of coccoid from rod forms closely resembled the morphological change undergone by *Bact. globiforme*, and the author considers strains of Group 2a to be probably related to this organism. This group is moreover believed to be related to *Corynebacterium*, while from their form members of Group 2b are considered to belong to Jensen's genus *Proactinomyces*. Though members of the motile Group 1 were not identified with known species the similarity in morphological and cultural behavior shown by strains of Groups 1 and 2 suggests a close relationship to the *Proactinomycetaceae*, motile species being recognized in both *Corynebacterium* and *Proactinomyces*. Gram-negative rods, classed in Group 3, were found to be less conspicuous than the Gram-positive forms. They were in the main chromogenic forms, and although they did not undergo the striking morphological changes exhibited by the Gram-positive types, they resembled the latter in their general biochemical inactivity.

In considering the evidence from qualitative studies so far reported a number of points seem to be established:

(1) In arable soils a large proportion of the bacterial flora consists of organisms whose functions are unknown or but little understood.

(2) Under normal soil conditions the predominant types of organisms are non-spore-forming short rods, motile or non-motile, cocci and spore-forming bacteria being relatively insignificant.

(3) Many predominant soil species are highly pleomorphic. Included in these are the *Bacterium globiforme* group and organisms closely related to corynebacteria and mycobacteria.

(4) The majority of soil bacteria are relatively inactive physiologically as judged by standard laboratory tests. This by no means excludes the possibility of important biological activity in soil.

The work so far done points to the value of more extensive studies of the qualitative nature of the soil microflora and the types predominating. Only when the autochthonous organisms are known more thoroughly will it be possible to learn their true functions in soil. Such knowledge is regarded as essential to an understanding of general microbiological activity in soil. In view of the intricate systems of symbiosis and antibiosis, it should help in evaluation of the known processes and form a basis for better appreciation of the relation of micro-organisms to growing plants, to soil borne plant diseases, and to soil fertility in general.

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

II. A SURVEY OF THE BACTERIAL FLORA OF SOILS DIFFERING IN FERTILITY[1]

By C. B. Taylor[2] and A. G. Lochhead[3]

Abstract

Investigations were made, on a non-selective basis, of the qualitative nature and relative incidence of the different types of the bacterial flora of three soils differing in fertility. The organisms were classified into eight groups. Non-spore-forming short rods, of which five groups were recognized, comprised nearly 90% of all types. Gram-negative short rods formed the most prevalent single group, rather more numerous than Gram-positive short rods. Gram-variable short rods, coccoid rods and pleomorphic rods *(Bact. globiforme)* were regarded as definite groups. Cocci, non-spore-forming long rods and spore-formers were less prominent soil types.

In spite of unequal productivity, the soils showed no outstanding differences in the relative incidence of the bacterial groups. Certain groups showed some indication of seasonal and cropping effect. The results suggest that the general character of the *autochthonous* (indigenous) soil flora is relatively uniform in soil of definite type, even though productivity may be greatly altered by manurial treatment.

The predominant soil bacteria appear relatively inactive in single culture. Moreover considerable divergence in biochemical action was shown by apparently closely related forms. It is suggested that the bacterial flora is relatively unstable physiologically, with considerable adaptability, and that the functions of the different species are exercised most fully only under conditions of association.

Introduction

The present paper is one of a number of studies on the qualitative nature of the microflora of soils, most of the relevant literature of which has been discussed in the first paper of this series (6). The object of the investigation was to study, on a non-selective basis, the bacterial types occurring in three soils, and their relative incidence. The soils were of similar type and crop history, but by reason of different fertilizer treatment for 25 years they had become widely dissimilar in productivity. A previous study (7) had been made of the abundance of certain strains of *Rhizobium* and *Azotobacter* in these soils, while as a side issue in the present work the incidence of *Bacterium globiforme* has been reported earlier (10).

Experimental

The soils studied were taken from three plots of different manurial treatment in a four-year rotation system of oats, clover, timothy and mangels. For the preceding 25 years the plots had been receiving the following treatments:

Soil N—no fertilizer

Soil X—15 tons farmyard manure, applied to mangels

Soil Y—100 lb. nitrate of soda, 300 lb. superphosphate, 75 lb. muriate of potash to mangels; 100 lb. nitrate of soda to oats, clover and timothy.

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[2] *Graduate Assistant.*
[3] *Dominion Agricultural Bacteriologist.*

The soils were of a sandy-loam nature and contained approximately 0.11%, 0.16% and 0.13% nitrogen respectively. The pH value fell between 7 and 8 with variations depending on crop, treatment and season. As may be noted from Table I, striking differences in fertility exist between treated and untreated plots. Mangel yields show this most clearly. The soils thus included an originally fertile soil impoverished by continuous cropping (N) and soils maintained at good fertility levels by farmyard and inorganic manures respectively (X and Y).

Composite samples were taken from the 2–4 in. layer, September 22, 1936, for preliminary tests, and on November 12, 1936, February 23, 1937, April 16, 1937, and July 21, 1937, from plots which had produced a crop of timothy and which at the time of the July sampling were supporting a mangel crop. In September and November, samples were likewise taken from corresponding plots after a crop of mangels. The February sampling represented frozen soil; but at the time of the April sampling the soil had thawed to a depth of 6 inches. Samples were analyzed as soon as possible. Plate cultures of 1/500,000 dilution were prepared. For comparison total cell counts were made by the ratio method of Thornton and Gray (12).

Cultural studies of the general microflora require a medium as non-selective as possible. For this reason, soil extract agar prepared according to Löhnis (8), and without added energy material, was chosen in preference to other media which, though synthetic and hence more easily reproducible, are regarded as more selective on account of the special energy sources contained. The advantage of soil extract was noted in previous tests (9), which gave higher counts with it than with more selective media such as Thornton's (11) mannitol-salts medium. From each sample five replicate plates were poured and incubated at 28° C. for 12 days before counting.

It is felt that the value of any qualitative study is enhanced when quantitative aspects are also taken into account. This is done not only by using definite dilutions, but by applying quantitative methods to an examination of the colonies. Haphazard selection or assumption of similar identity from microscopic observation are unsuited to an estimation of the relative incidence of different types. All colonies on a plate or on a definite sector should be examined on plates with a reasonable number of colonies. When few colonies are present the chances of error by regarding a possible contaminant as predominant are greatly increased. From representative plates all colonies on a sector containing approximately 60 bacterial colonies were picked and stab cultures made into soil extract semi-solid (containing 0.02% K_2HPO_4, 0.01% yeast extract and 0.3% agar). Preliminary tests indicated that many isolated strains, particularly from small colonies, refused to grow on various other media tested. The use of soil extract semi-solid not only assured the survival of 92% of the strains isolated but permitted a certain differentiation of type.

For group classification the cultures were examined for morphology and reaction to the Gram stain. To detect pleomorphic types of the *Bacterium*

globiforme group which appear first as rods and later change to cocci, observations were made on fresh transfers and on the same cultures at later stages of incubation. Standard agar was found to be an aid in the recognition of this group, though it was unable to support growth of many forms isolated. Physiological tests included gelatin liquefaction, nitrate reduction and dextrose utilization. For the nitrate reduction test semi-solid soil extract with 0.1% KNO_3 was used and for dextrose utilization soil extract semi-solid with 1% dextrose and indicator. The latter medium, being comparatively weakly buffered, was found to be more sensitive to changes than the usual media with peptone.

Plate and Total Cell Counts

A comparison of the three soils from the standpoint of "total numbers" is made in Table I in the form of summaries of the plate and cell counts. The usual seasonal fluctuation observed by so many previous workers is noted. The untreated soil appears less subject to fluctuation in numbers than the treated areas, particularly in cell counts. It is of interest to note that, although the plate count indicated that numbers were well maintained in the frozen (February) soil, cell counts showed in all three cases a drop from the November sampling. The results fail to show any relation between numbers and crop yield. In the case of the soils sampled after mangels particularly, there was little variation in numbers of organisms between the unfertilized and the fertilized areas, in spite of very large differences in crop-producing ability.

TABLE I

TOTAL CELL AND PLATE COUNTS

(millions per gram dry soil)

—	Following timothy			Following mangels		
	N	X	Y	N	X	Y
Yield (tons per acre) av. 25 years	2.01	3.10	2.66	7.99	22.72	20.91
Yield in 1936	1.65	2.95	2.51	2.59	29.03	25.12
Total cell count						
September	741.2	990.1	2065.9	2403.6	2380.7	2332.6
November	1144.5	1846.8	2524.9	2241.4	2534.5	2166.0
February	982.3	1524.9	1018.5	—	—	—
April	681.3	1071.5	925.3	—	—	—
Plate counts						
September	57.0	52.2	50.8	76.6	109.2	107.9
November	92.6	95.4	116.8	123.1	139.1	126.4
February	90.0	111.3	132.7	—	—	—
April	72.4	81.1	60.0	—	—	—
July	36.3	60.1	70.6	—	—	—

Main Morphological Groups

From the morphological and Gram-staining reactions of the cultures in semi-solid soil extract eight main subdivisions of the isolated organisms were made:

Group I Short rods, Gram-positive
Group II Short rods, Gram-negative
Group III Short rods, Gram-variable
Group IV Short rods, changing to cocci (*Bact. globiforme* group)
Group V Coccoid rods, Gram-positive
Group VI Cocci, Gram-positive or negative
Group VII Long rods, non-spore-forming
Group VIII Spore-forming rods

In some instances difficulty was found in allocating organisms to groups, particularly in the case of certain Gram-positive short rods where the differentiation between rod and coccus was nearly impossible. Such types have been grouped as coccoid rods. Subsequent physiological tests provided some justification for the separate grouping of these forms. Short rods that later became cocci and conformed generally to *Bact. globiforme* Conn have been classified under this head. Short rods that showed no tendency to form cocci were divided into three groups according to their reaction to the Gram stain. The percentage distribution of the various groups in the three soils following timothy and sampled at four seasons is shown in Table II. The cultural characteristics of the various groups are summarized separately for the three soils and given in Tables III, IV and V.

Short Rods

In line with findings reported by a number of previous workers (1, 5, 13) non-spore-forming short rods were found to comprise a large proportion of organisms capable of being isolated from soils. The five groups into which short rods were classified made up 86.7%, 89.6%, and 89.1% respectively of cultures isolated from Soils N, X and Y, a surprisingly close agreement in soils differing so widely in productivity.

Gram-negative short rods. Gram-negative short rods were found to be the most prevalent single group of organisms, being rather more numerous than Gram-positive short rods in each soil, taken over the course of the four sampling periods. The difference was less pronounced in Soil N than in the fertilized soils X and Y. Variation in relative numbers was noted at different seasons. Topping (13) reported the Gram-negative group to be much less frequent than Gram-positive types in soils studied by her. As is seen in Tables III to V this group is the least active physiologically and is suppressed to the greatest extent by modifying soil extract through the addition of dextrose, 42 of 226 strains being entirely inhibited by 1% concentration. It is possible that differences in proportion of groups found may be due to employment of different media for isolation. Some of those employed by Topping may be

TABLE II

MAIN MORPHOLOGICAL GROUPS AT DIFFERENT SEASONS

(Soils following timothy)

	November			February			April			July		
	N	X	Y	N	X	Y	N	X	Y	N	X	Y
Soil moisture, %	15.7	17.2	18.1	24.1	32.3	26.2	24.9	23.1	26.1	13.5	15.9	17.6
Total cultures isolated	59	61	61	62	62	63	64	64	64	41	43	41
Percentage showing no growth on transfer	8.5	13.1	13.1	6.5	3.2	6.4	6.3	3.1	17.2	0.0	11.6	4.9
Percentage showing growth on transfer	91.5	86.9	86.9	93.5	96.8	93.6	93.7	96.9	82.8	100.0	88.4	95.1
Morphological groups	%	%	%	%	%	%	%	%	%	%	%	%
Short rods, Gram-positive	24.0	28.3	35.8	39.6	46.6	30.5	28.3	17.7	9.4	24.4	28.9	20.5
Short rods, Gram-negative	37.0	26.4	32.0	25.8	15.0	35.6	26.6	53.2	56.6	34.1	50.0	46.1
Short rods, Gram-variable	18.5	18.8	9.4	3.4	10.0	13.5	3.3	11.3	15.0	2.4	0.0	0.0
Short rods, changing to cocci (*Bact. globiforme* group)	12.9	9.4	11.3	8.6	10.0	6.7	10.0	8.0	7.5	14.6	0.0	7.7
Coccoid rods, Gram-positive	0.0	0.0	0.0	8.6	11.6	6.7	18.3	4.8	5.6	4.8	5.2	2.5
Cocci, Gram-positive or Gram-negative	3.7	3.7	5.6	10.3	3.3	1.7	6.6	4.8	1.9	0.0	0.0	0.0
Long rods, non-spore-forming	3.7	9.4	5.6	3.4	3.3	5.0	5.0	0.0	1.9	0.0	2.6	2.5
Rods, spore-forming	0.0	3.7	0.0	0.0	0.0	0.0	1.6	0.0	1.9	19.4	13.1	20.5

TABLE III

CHARACTERISTICS OF BACTERIAL GROUPS

(Soil N—no fertilizer)

Groups	Total no. of cult.	Per cent of total	Gr. on N. A. v. sl. or abs., %	Gelatine		NO_3 reduction, %	Soil-extr. s. s. + 1% dextrose				No action, *gelatine*, NO_3 or dextrose, %
								Growth			
				Growth, %	Liquef., %		No gr., %	Acid, %	Alk., %	No ch., %	
Short rods, Gram-positive	63	29.6	54.0	57.1	25.4	49.2	3.0	53.9	20.6	22.5	11.1
Short rods, Gram-negative	65	30.5	50.8	56.9	13.8	15.3	13.8	24.6	26.1	35.5	35.4
Short rods, Gram-variable	15	7.0	100.0	33.3	0.0	86.6	0.0	20.0	13.4	66.6	13.4
Short rods, changing to cocci (*Bact. globiforme* group)	24	11.2	0.0	100.0	100.0	33.3	0.0	75.0	16.6	8.4	0.0
Coccoid rods, Gram-positive	18	8.4	88.8	38.8	16.6	61.1	11.1	38.8	27.7	22.4	16.6
Cocci, Gram-positive or negative	12	5.6	91.6	41.6	33.3	66.6	8.4	41.6	25.0	25.0	0.0
Long rods, non-spore-forming	7	3.3	57.1	85.7	14.3	14.3	14.3	28.5	28.5	28.7	42.9
Rods, spore-forming	9	4.2	11.1	66.6	44.4	44.4	11.1	66.6	0.0	22.3	22.3

TABLE IV

CHARACTERISTICS OF BACTERIAL GROUPS

(Soil X—farmyard manure)

Groups	Total no. of cult.	Per cent of total	Gr. on N. A. v. sl. or abs., %	Gelatine		NO_3 reduction, %	Soil-extr. s. s. + 1% dextrose				No action, gelatine, NO_3 or dextrose, %
				Growth, %	Liquef., %		No gr., %	Growth			
								Acid, %	Alk., %	No ch., %	
Short rods, Gram-positive	65	30.5	72.3	38.5	16.9	60.0	16.9	46.1	10.8	26.2	16.9
Short rods, Gram-negative	75	35.2	49.4	40.0	17.3	12.0	17.3	25.3	24.0	33.4	38.5
Short rods, Gram-variable	23	10.8	100.0	40.0	17.4	82.5	4.3	34.8	13.0	47.9	8.7
Short rods, changing to cocci (*Bact. globiforme* group)	16	7.5	0.0	100.0	100.0	25.0	0.0	75.0	12.5	12.5	0.0
Coccoid rods, Gram-positive	12	5.6	83.3	33.3	33.3	75.0	8.3	50.0	16.7	25.0	8.3
Cocci, Gram-positive or negative	7	3.3	100.0	57.1	14.3	85.7	0.0	42.8	14.3	42.9	0.0
Long rods, non-spore-forming	8	3.8	12.5	87.5	50.0	12.5	12.5	12.5	25.0	50.0	25.0
Rods, spore-forming	7	3.2	0.0	85.7	85.7	85.7	0.0	42.8	14.2	43.0	0.0

TABLE V

CHARACTERISTICS OF BACTERIAL GROUPS

(Soil Y—mineral fertilizers)

Groups	Total no. of cult.	Per cent of total	Gr. on N. A. v. sl. or abs., %	Gelatine		NO_3 reduction, %	Soil-extr. s. s. + 1% dextrose				No action, gelatine, NO_3 or dextrose, %
								Growth			
				Growth, %	Liquef., %		No gr., %	Acid, %	Alk., %	No ch., %	
Short rods, Gram-positive	50	24.5	84.0	48.0	18.0	48.0	14.0	46.0	10.0	30.0	30.0
Short rods, Gram-negative	86	42.1	64.0	48.6	19.7	5.0	23.2	37.0	19.7	20.1	38.3
Short rods, Gram-variable	21	10.3	100.0	47.6	33.3	66.6	4.8	42.8	23.8	28.6	4.8
Short rods, changing to cocci (*Bact. globiforme* group)	17	8.3	0.0	100.0	100.0	52.9	0.0	82.3	0.0	17.7	0.0
Coccoid rods, Gram-positive	8	3.9	100.0	50.0	25.0	87.5	0.0	50.0	12.5	37.5	0.0
Cocci, Gram-positive or negative	5	2.4	80.0	40.0	20.0	20.0	20.0	0.0	20.0	80.0	60.0
Long rods, non-spore-forming	9	4.4	33.4	55.5	11.1	11.1	22.2	33.3	22.2	22.7	22.2
Rods, spore-forming	8	3.9	0.0	100.0	75.0	25.0	12.5	50.0	12.5	25.0	12.5

considered as fairly selective while there is no indication of what proportions of the organisms studied by her originated on the several media used.

Gram-positive short rods. Gram-positive short rods were the second most abundant group in all soils. This group displayed rather more activity than Gram-negative forms as judged by ability to reduce nitrates, liquefy gelatine or utilize dextrose. Like the latter group, however, a considerable percentage showed no growth on ordinary gelatine or agar and are believed to represent largely forms indigenous to soil only, brought out by such media as soil extract.

Gram-variable short rods. Gram-variable short rods appeared to comprise a definite group failing to give a uniform reaction to Gram staining, though both Hucker's and Kopeloff's modifications were used. Though numerically less important than either of the above groups they showed certain characteristics which presumably justified their being classified separately. None of 59 strains isolated was able to grow on nutrient agar while their most pronounced biochemical feature was their comparatively high nitrate-reducing ability.

Cocci-forming rods. Cocci-forming rods classified as the *Bacterium globiforme* group were an important group in all soils studied, comprising 11.2%, 7.5% and 8.3% of the organisms isolated from Soils N, X and Y respectively. Members of this group, though definite rod forms in young cultures, show a change to the coccal form with longer incubation. As previously indicated (10) variations in cell size and rate of change from rod to coccus are noted between different strains of this group. Physiological tests further emphasized differences which may be exhibited by apparently closely related strains. Thus in a separate experiment in which 50 cultures of *Bact. globiforme* were compared as to ability to utilize six sugars, hydrolyze starch and reduce nitrates, 40 actual variations in physiology were noted. As a group these organisms were the most active of those found, and were uniform in ability to liquefy gelatine and grow on standard media.

Coccoid rods. Coccoid rods, representing Gram-positive short rods that could not be satisfactorily differentiated from cocci, produced 8.4%, 5.6%, and 3.9% of the organisms isolated from Soils N, X, and Y. Like the Gram-variable short rods, the great majority failed to grow on nutrient agar, and included a large proportion of nitrate-reducing forms.

Cocci, Long Rods and Spore-formers

These three groups represent less prominent soil bacteria, judging from their abundance in the soils studied. Cocci comprised 5.6%, 3.3% and 2.4% of the strains isolated from the three soils. Conn (1, 2) found cocci to be numerically insignificant, and inclined to the belief that they are not to be regarded as characteristic of soil. In the soils studied by us the cocci isolated showed much variation in type and appeared to represent a variety of species each present in but small numbers.

Non-spore-forming long rods. This group comprised 3.3%, 3.8% and 4.4% of the total organisms isolated from the three soils, while spore-forming

rods were found to the extent of 4.2%, 3.2% and 3.9%. Only in the case of the July sampling did the last-named group represent an appreciable percentage of the forms isolated.

Discussion of Predominant Forms

A close comparison with the short rod forms described by Conn (2, 3) and Topping (13) is made difficult on account of the use of different media, making for differences in grouping and in estimating relative abundance in soil. Thus Gram-negative rods were of greater significance than is indicated by the work of these authors. However it appears that the non-spore-forming short rods comprising our Groups I to V correspond in large measure to types of short rods described by Conn and particularly to those classed as "slow growers" or "punctiform colony forming" organisms. Such terms may be misleading in some instances, since organisms producing little growth on a comparatively deficient medium, such as tap-water gelatine, may grow profusely on other media. This has been found to be the case with organisms included in our *Bact. globiforme* Group IV.

It appears that Conn's Group I, comprising simple rods, includes types which we have subdivided into our Groups I, II, III, and V. On the other hand our *Bact. globiforme* group is rather more inclusive than Conn's, embracing not only his Group II *(Bact. globiforme)*, comprising forms showing change from rod to coccus, but also his less abundant Groups III and IV, characterized by a tendency to produce branched forms. The last-named groups are doubtless related to pleomorphic, coccus-forming types forming "sprouts", or branching forms described by Topping, who suggests a close relationship between her Groups 1 and 2 and *Bact. globiforme*. Belief in this relationship is strengthened by observation of strains of *Bact. globiforme* including one obtained from Dr. Conn which show ability, especially in liquid media, to produce branching forms characteristic of Conn's Groups *III* and *IV* and Topping's pleomorphic groups. Moreover comparison of Figs. 11, 5, 3 and 15 given in Topping's paper with Figs. 1, 2, 3 and 5 respectively in the paper of Taylor and Lochhead (10) on *Bact. globiforme* suggests that these authors worked with very closely related forms. The apparently higher incidence of pleomorphic types comprising *Bact. globiforme* and related forms found by Conn and Topping as compared with our present studies is believed to be due largely to the use of media more selective for these forms. The selective action of tap-water gelatine as compared with soil extract agar has been previously demonstrated (10).

Relation of Bacterial Groups to Soils Studied

In Table II, giving the incidence of the different morphological groups in the three soils at four sampling dates, no outstanding differences are seen between the unfertilized soil, N, and the fertilized soils, X and Y, in spite of great variation in crop-producing capacity. The uniformity is the more interesting since Soil X received an application of farmyard manure three

weeks previous to the November sampling. Slightly higher percentages of non-spore-forming long rods and of spore-formers were noted in this soil but otherwise the group incidence approximated closely that of Soils N and Y. In line with the findings of Joffe and Conn (4) it is apparent that if notable increases in spore-formers are to result from addition of organic materials, amounts in excess of normal field applications are needed.

Throughout the tests there was no indication that the incidence of *Bact. globiforme* bore any relationship to the productivity of the soils examined, nor was it possible to correlate fertility with the relative abundance of any of the other main groups into which the organisms were classified. The soils in question, originally the same, had become different in crop-producing ability by artificial means, and the results suggest that in a soil of given type the bacterial flora may be fairly resistant to change, even though productivity may vary as much as ten-fold.

There is indication of rather more difference in group incidence due to cropping than between the soils themselves. Comparative data, following timothy and mangels respectively, are given in Table VI, in which the approximate numbers in millions per gram dry soil are shown. After mangels, increased counts of Gram-positive and Gram-variable short rods and *Bact. globiforme* were found, as compared with the numbers following timothy. Mangels in general supported a higher bacterial population as measured by both total and plate counts (Table I).

TABLE VI

BACTERIAL GROUPS IN THREE SOILS FOLLOWING DIFFERENT CROPS

Morphological group	Millions per gram of dry soil					
	Following timothy			Following mangels		
	N	X	Y	N	X	Y
Short rods, Gram-positive	20.0	22.3	34.6	28.7	55.7	44.1
Short rods, Gram-negative	30.8	20.9	30.9	16.4	23.2	27.3
Short rods, Gram-variable	15.4	14.9	9.1	24.6	16.2	14.7
Short rods, changing to cocci (*Bact. globiforme* group)	10.7	7.4	9.1	18.4	13.9	14.7
Cocci, Gram-pos. and Gram-neg.	3.1	3.0	5.5	8.2	0.0	12.6
Long rods, non-spore-forming	3.1	7.4	5.5	8.2	6.9	2.1
Rods, spore-forming	0.0	3.0	0.0	4.2	0.0	0.0

The effect of season on the relative incidence of the different bacterial groups was in general not marked (Table II). The most pronounced changes occurred in the July sampling, which showed a decrease in Gram-variable short rods and a rather notable increase in spore-formers in all soils. At this sampling the mangel crop was growing but no definite reason for the degree of prominence of this latter group is offered.

Conclusion

Micro-organisms in the untreated soil, N, might be expected to represent for the most part the *autochthonous* microflora, *i.e.*, the indigenous group of

organisms postulated by Winogradsky (14, 15) as contrasted with the *zymogenic* group, composed of forms relatively scarce in normal soils, but becoming active upon additions of readily decomposable substances. This latter group might be supposed to be more evident in Soil X, receiving farmyard manure. As judged from the grouping of the bacteria isolated, however, no evidence of alteration of types was found, suggesting that the autochthonous flora of the soil type studied was little affected by the treatments given. Whether differences exist that are not brought out by general grouping, would have to be decided by studies of a much more specific nature.

More detailed study of strains isolated, however, particularly physiological tests, showed a surprising variability in apparently very closely related types. In each of the main groups identity of characteristics was the exception rather than the rule, with each additional test bringing out slight divergencies. The degree of biochemical activity displayed by the predominant soil organisms was regarded as relatively low. It is suggested that the indigenous bacterial flora is comparatively unstable physiologically and possessed of considerable adaptability. The comparative inactivity of so many forms when isolated from soil and cultivated singly also suggests that the functions of these species are exercised most fully only when they are acting in association with other micro-organisms. In aiding toward a better understanding of these functions research with mixed cultures will doubtless play an important part. The limitations attendant on an investigation of one type of soil, as in the present study, are recognized, and it is hoped to extend the work to a variety of fertile and infertile soils of different types.

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THE TRANSFERENCE OF DURUM AND DICOCCUM CHARACTERS TO 21-CHROMOSOME WHEAT LINES BY CROSSING[1]

By T. J. Arnason[2]

Abstract

Hybrids between *vulgare* ($n = 21$) and emmer ($n = 14$) wheats were backcrossed to *vulgare*, and segregates having the *vulgare* chromosome number were studied with respect to their emmer characters. Eleven of the 24 characters used in a *vulgare-durum* cross and 6 of the 23 characters used in a *vulgare-dicoccum* cross appeared in emmer condition in hybrids. Most of the other characters differed from the *vulgare* form in a number of segregates. From a comparison of the results of this investigation and a previous one dealing with 14-chromosome segregates, it is concluded that the *vulgare* form of many characters is due to genes in the primary chromosome sets A and B, the *vulgare* form of many others to genes in both the primary sets and the secondary set C.

Introduction

The transmission of some *vulgare* characters to stable 14-chromosome* segregates was discussed in a paper by Thompson, Arnason and Love (1). The account that follows deals with the transfer of *durum* or *dicoccum* characters to 21-chromosome lines derived from *vulgare-durum* and *vulgare-dicoccum* crosses.

Twenty-one-chromosome segregates of a *vulgare-durum* or *vulgare-dicoccum* cross presumably can have any combination of *vulgare* and *durum* or *dicoccum* chromosomes from the primary sets A and B which mate in F_1 together with the complete C set of *vulgare* which lack mates in F_1. By repeated selfing in several lines many plants homozygous for a few *durum* or *dicoccum* genes should be obtained. Observations on large numbers of plants from many lines should show then whether the genes from emmer series plants can produce effects in 21-chromosome plants similar to those they produce in 14-chromosome ones. When such an effect is obtained, *i.e.*, when a character appears in *durum* or *dicoccum* condition, the *vulgare* genes in the C set have not apparently affected its expression. If the character in question has been reported in *vulgare* form in 14-chromosome plants it may then be concluded that no genes of importance affecting the character are present in the C set. If the *vulgare* form has not appeared in 14-chromosome plants, its production probably depends on interaction between genes in the primary sets A or B with C-set genes or upon multiple factors, some of which are in the C set.

Materials and Methods

The parental strains were Marquis (V), a *vulgare* wheat, Iumillo (D), a *durum*, and Vernal, (E), a *dicoccum*. VD and VE plants, F_1 generation, were backcrossed to Marquis. The backcross (F_2) plants (V-VD and V-VE)

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Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan.
[2] *Assistant Professor of Biology, University of Saskatchewan.*
* *Haploid chromosome numbers are used throughout.*

were selfed, as were their progeny. Some backcross lines have been carried to F_6, others to F_4 or F_5 only. A number of stable lines ($n = 21$) have been obtained, a few lines are still unstable and a few have reverted to the 14-chromosome condition.

Chromosome counts were made on pollen mother cells from a total of 294 F_4, F_5, and F_6 plants. Most of these counts were made on smeared material, but some were made from permanent slides fixed in Karpechenko's modification of Navashin's fixative, and stained with crystal violet and iodine. The complete count could not always be made. In such cases where many cells had no univalents, the plants were interpreted as having 21 bivalents. Occasionally rings of four chromosomes or chains of three were encountered, but these were rare.

Characters Studied

The backcross V-VD plants were examined to determine the condition of 24 morphological characters; the V-VE plants were examined for the condition of 22 characters. Brief descriptions of the characters and their condition in the parental varieties are given below. The descriptions are based in each case on about 100 observations or measurements.

NOTE: v = *vulgare*, d = *durum*, e = *dicoccum*

1. Stem diameter—measured at 2 cm. below the collar; v, 1.6–2.5 mm.; d, 1.1–1.6 mm.; e, 1.1–1.5 mm.
2. Stem cavity—observed 2 cm. below collar; v, large cavity, thin walls; d and e, no cavity.
3. Collar—at base of spike; v, $\frac{3}{4}$ of stems have open collar; d, all closed; e, $\frac{1}{8}$ open.
4. Head length—measured from the base of the lowest spikelet to the tip of the terminal one; v, 7.5–11.0 cm.; d, 5.5–7.5 cm.; e, 6.1–8.4 cm.
5. Density of spike—calculated by dividing the head length by the spikelet number; v, 4.7–6.3; d, 3.5–4.7; e, 3.6–4.7.
6. Head form—width of the 1-ranked side divided by the width of the 2-ranked side at the centre of the spike; v, 1.2–1.6; d, 0.8–1.1; e, 0.5–0.8.
7. Glume adherence; v and d, loose; e, tight.
8. Glume length, exclusive of glume tooth; v, 7.8–9.0 mm.; d, 9.0–10.3 mm.; e, 9.1–11.0 mm.
9. Glume shape—empty glume; v, wide at top and bottom, broadest near base, blunt; e and d, broadest near the middle, tapering to both ends.
10. Glume cross-section taken near the centre of the glume; v, broad U-shaped; d, V-shaped; e, nearly V-shaped.
11. Keel prominence; v, prominent but not deep and sharp as in d and e.
12. Keel hairs; v, sparse stout hairs on upper half of keel, fine or none on lower half; d, very fine, closely placed hairs from tip to near base; e, few, usually about 10, stout hairs near the tip.

13. Glume tooth; v and e, short terminal tooth 1 mm. or less in length; d, long tooth, 2–3 mm.
14. Tooth sharpness; v, blunt; d, sharp, fine point; e, rather dull, coarse point.
15. Beard length—measured on middle spikelets; v, up to 1 cm.; d, 10–12 cm.; e, 10–13 cm.
16. Glume shoulder; v, wide, nearly horizontal; d and e, narrow, sloping down sharply.
17. Rachis width—1—measured on central segments at widest point; v, 2.5–3.2 mm.; d, 2.0–2.4 mm.; e, 1.1–1.8 mm.
18. Rachis width—2—measured at the narrow end. In all cases the lower end is the narrow end; v, 1.7–2.3 mm.; d, 1.3–1.6 mm.; e, 0.7–1.3 mm.
19. Rachis shape; v, curving, widest point a short distance below the top of the segment; d and e, sides straight, segment widest at the top.
20. Rachis hair tuft—the hairs at the centre of the rachis face, between the bases of the empty glumes; v, few, sparse, rather short; d, single large tuft of long hairs; e, small compact tuft of long hairs.
21. Rachis hairs along sides; v, long hairs uniformly and rather sparsely distributed from top to bottom; d, fine hairs closely placed, long at top, short near base of rachis segment; e, sides bare.
22. Rachis hairs in upper corners; v, few long hairs; d, dense tuft of long hairs; e, none.
23. Rachis fringe—hairs along the top of the rachis segments (except the central tuft); v, few short scattered hairs; d and e, none.
24. Rachis articulation; v and d, tough; e, brittle, breaks at base of rachis segments.
25. Grain length; v, 5.0–6.2 mm.; d, 6.0–7.5 mm.; e, 7.0–9.0 mm.
26. Grain hairs; v and e, long, many; d, short, few.
27. Leaf hairs—upper side of young leaves; v, sparse, moderately long hairs at crests of ridges, few short hairs on sides; d, no hairs; e, many long hairs uniformly distributed.

Durum and *Dicoccum* Characters Appearing in 21-chromosome Segregates

Pollen mother cells of 198 V-VD plants representing 12 lines, and 96 V-VE plants representing 7 lines were examined. Of these, 86 V-VD and 22 V-VE plants, representing 9 and 3 lines respectively, had 21 pairs of chromosomes. As used here a line includes all the descendents of an F_2 (*i.e.*, a second generation backcross) plant. The *durum* and *dicoccum* characters which have appeared apparently unchanged in 21-chromosome plants are listed in Table I. It will be observed that fewer *dicoccum* than *durum* characters were transferred to 21-chromosome plants. This may be due, in part, to the fact that fewer 21-chromosome *dicoccum* hybrids were found. The smaller number of these was due to the fact that in *dicoccum* hybrids cytological irregularities were

TABLE I

CHARACTERS FOUND IN *durum* OR *dicoccum* CONDITION IN 21-CHROMOSOME SEGREGATES

Character	*Vulgare-durum* hybrids		*Vulgare-dicoccum* hybrids	
	No. of plants	No. of lines represented	No. of plants	No. of lines represented
Stem diameter	5	3		
Collar	5*	4		
Head length	2	2		
Head form	21	8	2	1
Glume length	11	5		
Glume tooth	7	3		
Tooth sharpness	29	4		
Shoulder	13	4	5	2
Grain length	17	9	2	2
Rachis fringe	36	6	6	3
Rachis hairs in upper corners	12	4		
Glume adherence			4	2
Rachis hair tuft			6	2

* *Failed to breed true.*

eliminated with greater difficulty than in *durum* hybrids. Some of the differences in the results from the two crosses may be attributed to genetic differences between *durum* and *dicoccum*. This is obvious in cases in which one of the emmer species resembles *vulgare* with respect to a character, while the other species differs, *e.g.*, the rachis articulation character.

Characters which failed to appear in completely emmer condition but did appear in intermediate condition are listed in Table II. Eight characters

TABLE II

CHARACTERS FOUND IN INTERMEDIATE CONDITION IN 21-CHROMOSOME PLANTS

Character	*Vulgare-durum* hybrids		*Vulgare-dicoccum* hybrids	
	No. of plants	No. of lines represented	No. of plants	No. of lines represented
Cavity of stem	8	3	1	1
Density	1*	1		
Glume cross-section	3	3	1	1
Glume shape	32	7		
Keel prominence	24	5		
Keel hairs	18	5		
Beard length	30	6	14	3
Rachis width-1	3†	2		
Rachis width-2	2†	2		
Rachis curve	13†	4	12†	4
Rachis hair tuft	3	3		
Grain hairs	12	3		
Rachis articulation			8	4

* *Progeny all had v density.*

† *Shift from vulgare very slight.*

appeared in intermediate condition in *vulgare-durum* hybrids; four in *vulgare-dicoccum* hybrids. The rachis width and curve characters mentioned in the table were very near *vulgare* in all cases and the *durum* density recorded for one plant may have been brought about by environmental factors, since the character did not appear in any of the progeny of this plant.

Certain characters appeared in exaggerated *vulgare* form in some segregates. These V+ or super-*vulgare* characters are listed in Table III. Five of these were in *vulgare-durum*, three in *vulgare-dicoccum* hybrids.

TABLE III

V+ CHARACTERS IN 21-CHROMOSOME LINES

Character	*Vulgare-durum* hybrids		*Vulgare-dicoccum* hybrids	
	No. of plants	No. of lines represented	No. of plants	No. of lines represented
Head length	12	4	3	1
Density of spike	18	7	9	3
Head form	11	5	2	1
Rachis width-1	5	3		
Rachis width-2	1	1		

The Mode of Inheritance of Some Characters

Some of the *vulgare* characters that have been observed in 14-chromosome segregates (1) have their *durum* and *dicoccum* counterparts in 21-chromosome plants. Such characters are stem diameter, glume length, tooth length, tooth sharpness and glume adherence. All the genes necessary for the production of the *vulgare* form of these characters must lie in the A and B sets of chromosomes. A number of other characters such as head length, glume shoulder, rachis fringe, rachis hair tuft and seed length may appear in *durum* or *dicoccum* condition in 21-chromosome plants, but it is doubtful whether the *vulgare* condition can be obtained in 14-chromosome plants. It is possible that in such cases *durum* and *dicoccum* genes are epistatic to certain genes in the *vulgare* C set. This may be illustrated by the observation made in the case of glume shoulder characters. No 14-chromosome plant was credited with a completely *vulgare* glume shoulder, though some approached this form. The *durum* condition occurred in 21-chromosome plants. Hence it appears likely that *vulgare* has genes influencing the shape of the glume shoulder in the primary sets (A or B) and in the secondary set (C). *Durum* genes must then be epistatic to the *vulgare* C genes.

The character called head form may be affected in several ways, for example by glume length, glume width, glume adherence and the plumpness of the seeds. Since a numerical ratio of width to depth only is taken, plants listed as having similar head form are not necessarily genetically alike with respect to the character, as similar ratios may be brought about by different combinations of glume and other spike characters. Also, since the character is

expressed in terms of a ratio, the absolute measurements may differ from those of the parent which has a similar head form. As might be expected, some factors affecting head form are believed to lie in the C set of chromosomes in spite of the fact that the *durum* and *dicoccum* forms do appear in 21-chromosome segregates. In 14-chromosome derivatives of a *vulgare-dicoccum* cross *vulgare* head form was not observed (1). All the 21-chromosome plants having *dicoccum*-type head form also had tightly adhering glumes and long rachis segments.

Characters which never appeared in *durum* or *dicoccum* condition in 21-chromosome segregates and which are, therefore, presumed to be conditioned by C-set genes, include the following: stem cavity, density of spike, rachis width, rachis shape, rachis articulation (toughness), glume shape, glume cross-section, keel prominence, keel hairs, beard length, rachis hair tuft, rachis hair sides, leaf hairs. Some of these, and one additional character, will be dealt with individually.

In the *durum* variety used all the collars were closed. The *vulgare* variety was less constant. Usually in a plant having several tillers most of the collars were open, but often a few were closed. A count of 100 *vulgare* stems gave a ratio of three-fourths open to one-fourth closed. Some of the 21-chromosome hybrid segregates had a smaller proportion (one-half) of open collars than had *vulgare*. A few plants with all the collars closed were found but in every case each of the progeny had some open collars. The conclusion arrived at in an earlier paper (1) that the open collar of *vulgare* is dependent on C-set genes may be correct but it appears that genic control of this character is not complete.

Previous work (1) has shown that the near-beardless condition of *vulgare* is due to genes in the primary chromosome sets. Many 21-chromosome segregates had long beards but not as long as in the *durum* or *dicoccum* parents (1 to 4 cm. shorter). Therefore, it is concluded that in the C set one or more beard-modifying factors are present.

While no 21-chromosome derivative of the *vulgare-dicoccum* cross had as brittle a rachis as *dicoccum*, many were more brittle than *vulgare*. In addition, some segregates having less than 21 bivalents were more *dicoccum*-like in that respect, confirming the view that genes for tough rachis are present in the *vulgare* C set.

Vulgare has long sparse hairs on the ridge tops of the leaves and few short hairs on the ridge sides. *Durum* has no hairs and *dicoccum* has many long hairs uniformly distributed on the ridge tops and sides. All the 21-chromosome hybrids from both crosses had long ridge-top hairs though there was some variation in their number and length. In the *dicoccum* cross long hairs also occurred often on the ridge sides. These side hairs were not as long as some top hairs. In *durum* hybrids long hairs never occurred on the ridge sides, and the top hairs, though classed as long, were considerably shorter than in *dicoccum* hybrids. *Vulgare*-type leaf hairs were previously reported as occurring in several 14-chromosome *vulgare-dicoccum* hybrids and one *vulgare-*

durum hybrid (1). The facts suggest that two or more genes affecting leaf hairs are present in *vulgare*. Probably at least one gene influencing especially ridge-top hairs lies in the C set.

The inheritance of rachis hair characters is not clear. Since many 21-chromosome segregates had no fringe across the top, it is concluded that genes for fringe hairs are lacking from the C set. The *vulgare* type of rachis hairs at the sides occurred in all *vulgare-dicoccum* segregates. A gene for these hairs is probably in the C set; another such gene is then probably in A or B since "near-*vulgare*" rachis hairs were reported for some 14-chromosome segregates (1).

The exaggerated *vulgare* characters in the V+ group must depend in part on the genes of the C set of chromosomes, since these characters never appear in 14-chromosome segregates. The V+ characters may be due to multiple factors, to interaction between *durum* or *dicoccum* and C set genes, to gene duplication or to polysomy. In the absence of critical cytological evidence a decision cannot be made between the alternatives.

The sharp tooth of the empty glume, a characteristic of the *durum* variety, appeared in a number of hybrids. In other segregates the tooth was extended to form a short awn. Similar short awns were observed also in 14-chromosome segregates (1). The awned condition was always associated with beards though the shorter *durum*-type tooth appeared occasionally in beardless plants. Watkins has reported linkage between beards and glume-tooth awns (2). The absence of awns in the bearded parental varieties must be due to awn-suppressing genes. The awned segregates lack the suppressors. There is no evidence that C-set genes affect the expression of this character.

Discussion

The *vulgare* form of some characters did not appear in any of the 14-chromosome segregates examined by Thompson, Arnason and Love (1). Of this group of characters, those which are always *vulgare* in 21-chromosome segregates, *e.g.*, the rachis width characters, must owe that form to C-set genes. However, the *vulgare* form of some of these characters, *e.g.*, head form, does not occur in all 21-chromosome segregates. For such characters C-set genes, together with certain A- or B-set genes, are necessary.

In one case it has been shown that the genes necessary to produce the *vulgare* form are not all the genes that may affect the character. The *vulgare* beardless condition is easily transmitted to 14-chromosome segregates, but the long beards of emmer were not found unmodified in 21-chromosome plants. Presumably beard-modifying factors are present in the C set. In normal beardless *vulgare* these factors would have no visible effect.

In general, conclusions arrived at earlier, with respect to the location of genes responsible for *vulgare* characters, are supported by the results reported here. The present study has shown also some of the variations of form that may be brought about by shuffling together primary-set genes of emmer and *vulgare* and dealing out new "hands", including in each a complete C set.

If, as seems probable, *vulgare* wheat is an allopolyploid, in which the C set of chromosomes came from a plant whose haploid chromosome number was 7, then originally this set must have contained genes affecting all parts of the plant. How far the integrity of this or any set of chromosomes has been maintained is not known. It is not unlikely that some rearrangement of chromatin has taken place both within and between chromosomes.

Doubtless the original allopolyploids had many of the characters, or combinations of characters, that occur in modern members of the *vulgare* group; other character modifications probably appeared as a result of gene mutations and gene losses. But these presumably would be equally likely to occur in any chromosome. For this reason differentiating factors might be expected to be scattered, not confined to any one "set" of chromosomes. This appears to be the case in our material. The *vulgare* form of some structures is determined mainly by genes in the A and B sets, of others by genes in the C set, still others require genes from the primary sets (A and B) and the secondary set (C) for the production of the typical *vulgare* form.

Characters which are confined to 21-chromosome lines of wheat must depend on genes in the 7 chromosomes of *vulgare* which do not pair with members of the "emmer series" chromosomes in hybrids. All others can appear in 14-chromosome plants. The known number of "group distinguishing" characters is quite small. Most *vulgare* characters are not distinguishing, in the sense that they do not occur in any 14-chromosome plants (2).

Conclusions

The primary sets A and B, of Marquis wheat *(vulgare)* differ in many genes from those of Iumillo *(durum)* and Vernal *(dicoccum)*. Some of these *vulgare* genes produce typical *vulgare* characters in 14-chromosome plants, but some do not. Similarly some of the "emmer series" genes produce typical "emmer" characters in 21-chromosome plants, while others do not.

Genes of the C set affect many characters, and must affect all those which can not be obtained in *vulgare* form in any 14-chromosome segregates. The many "emmer series" characters that fail to appear unchanged in 21-chromosome plants must owe their modification in many cases to C-set genes. It is quite possible that more of these characters than have been reported here can be transferred unchanged. This is especially true of derivatives of the *vulgare-dicoccum* cross in which the group of determined 21-chromosome plants was very small.

Acknowledgments

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STUDIES ON BLACKHEART, SOFT-ROT, AND TARNISHED PLANT BUG INJURY OF CELERY[1]

By J. K. Richardson[2]

Abstract

A study of blackheart, soft-rot and tarnished plant bug injury on celery was made to determine their interrelationship, in addition to their individual effects on the host. The incidence of blackheart could not be correlated with a saturated condition of the soil, or with the use of various fertilizers, but under artificial conditions the disease could be induced in susceptible plants by subjecting them to temperatures ranging from 85–95° F., in a humid atmosphere. Experimental data supplemented by observations in the field indicate (i) that the disease is physiological in nature, (ii) that early plantings are more severely affected, (iii) that most extensive injury occurs when plants are nearing maturity, (iv) that vigorous plants are more subject to attack, (v) that there is a difference in varietal susceptibility, and (vi) that the appearance of the disease in the field is generally preceded by a period of high humidity or of high temperature, or of both.

In addition to the soft-rot caused solely by *Erwinia carotovora* (L. R. Jones) Holland, necrotic blackheart tissues under favorable conditions often become infected by this pathogen, which, as a secondary decay, destroys the plant.

The tarnished plant bug *Lygus pratensis* L. is of economic importance as a vector of soft-rot. Considerable damage, differing in appearance from either blackheart or soft-rot, may also be caused by its feeding habits.

Introduction

In some seasons, blackheart is extremely prevalent and destructive in certain districts of Ontario. Much of the celery in the province is grown in the market-gardening districts where a variety of soil types and cultural practices obtain. Most growers in these localities have suffered severe losses from this disease, but when questioned, could supply only meagre information concerning the trouble.

The disease has been attributed to unsuitable environmental conditions, infection by *Erwinia carotovora*, attacks by certain insects, or a combination of two or more of these factors. The present studies were undertaken to investigate the relative importance and possible interrelations of the various factors involved in blackheart of celery in Ontario.

Review of Literature

The first reference to a heart rot of celery was made by Halstead in 1892, when he described a bacterial disease occurring in New Jersey, which closely resembled soft-rot of carrots. The following year Beach (2) described two troubles present in New York State, one of which was a typical soft-rot, the other a withering of the leaflets and decay of the stalks, although both conditions may have been symptoms of the same disease. The cause of the trouble was not determined, but it was more prevalent during the summer months and progressed most rapidly when conditions were moist and hot.

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Contribution No. 548 from the Division of Botany, Experimental Farms Branch, Dominion Department of Agriculture, Ottawa.
[2] *Assistant Plant Pathologist, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario.*

In a favorable environment, however, plants that were not too badly attacked were able to form new hearts. Kinney (9) reporting a similar disease in Rhode Island in 1897 named it "blackheart", stated that it was widespread and not confined to any particular varieties, that it was not seed-borne, that it occurred on both irrigated and non-irrigated land, and that it appeared to be worse after periods of high temperature. Winters (16) in Florida in 1907 and 1908, conducted extensive research on blackheart and came to the conclusions, (i) "that some physiological weakness produced bleaching, which made the plants susceptible to infection by bacteria; (ii) that certain fertilizers increased the susceptibility of plants to the disease; (iii) that bacteria were not the direct cause of blackheart." In 1915 (1) a disease similar to that occurring in Florida was reported from Bermuda. In England in 1914 and 1916, Wormald (17, 18) gave a complete description of soft-rot of celery caused by a strain of *E. carotovora*. Poole (13) stated that in New Jersey investigations were being carried on with stem and root rot of celery in 1917, and in 1921 (14) he reported severe losses due to bacteriosis in the same state. In the same year Smith (15) reported that in the delta region in California, a celery crown rot was found to be due to the soft-rot organism *E. carotovora*. Foster and Weber (5) in 1924 discovered that the blackheart prevalent in Florida was not influenced by fertilizers, but could be induced at will by flooding plants by means of sub-irrigation. In 1932, Foster (4), reporting the results from a questionnaire distributed throughout the United States and Canada, states that while blackheart is sometimes confused with bacterial soft-rot, the latter causes a decay of the affected parts without hope of recovery, whereas the former, which originates in the heart of the plant, is frequently outgrown.

The relation of insects to blackheart and soft-rot has received considerable attention. In 1917 Hearst (7) reported a rapid bacterial heart decay of celery plants which was associated with, and secondary to, the injury of young shoots caused by the insect *Lygus pratensis* L. Ten years later Leach (10) described a bacterial heart rot which was spread in the field by larvae of the dipterous leaf miners *Scaptormyza gramineum* Fall., and *Elachiptera costata* Leow. In England in 1934, Ogilvie (11) associated soft-rot with the injury of carrot flies, slugs and the drosophilid, *S. gramineum* Fall.; and in Ontario Caesar (3) reported that the insect *L. pratensis* L. kills the tips of the inner leaf stems of the celery plant, thus enabling bacteria to enter and destroy the heart.

Blackheart

Symptoms of the Disease

The initial symptoms of blackheart develop suddenly on the youngest heart leaves as a discoloration and water-soaking, followed by necrosis of the tips, margins, veins or entire leaf blades. Frequently there is no further progress of the disease and the plant continues to develop normally with affected leaves remaining as black, shrivelled, necrotic tissue at the tips of elongated stems. In cases where the attack is slight, plants may outgrow the disease several times during the season. In severe cases, however, the unopened heart tissues as well as the youngest leaves are destroyed, and such plants often show an

outer fringe of normal leaves surrounding a crown of short stalks, tipped with black, leathery necrotic tissue. After the initial appearance of blackheart, plants may be totally destroyed by bacteria which gain entrance through the physiologically produced necrotic leaf tissues.

During the progress of the present investigations it was observed that blackheart developed with greater regularity in plants in the greenhouse than in those growing under field conditions; but regardless of environment, the disease usually appeared when the plants were reaching maturity. Younger plants occasionally developed slight symptoms, but when affected at this early stage they usually recovered and continued to grow normally.

Field Observations

During 1933 and 1934, careful records were taken of soil types, cultural practices and growing conditions on a number of farms where outbreaks of blackheart had occurred. The disease was not general, but when present, it usually appeared during late July or August in crops which had been planted early. Large, vigorous plants were always the most seriously affected.

Experiments

Effect of Soil Moisture

Although blackheart of celery has been induced by flooding (5), field experiments conducted over a period of five years with some 1200 plants at various stages of development in soils saturated by various methods, have produced negative results.

In addition, three greenhouse experiments involving 420 plants were conducted at different seasons of the year. Each included three groups of plants, the first watered daily, the second whenever the soil appeared dry, and the third when the plants began to wilt. In the several experiments the number of plants which became diseased ranged from 70–92% in the first group, 35–70% in the second, and 5–54% in the third. In addition, the heavily watered, more vigorously growing plants were most severely affected and rarely recovered, while in the sparingly watered groups recovery was general.

In another experiment 100 young celery plants were set in seven-inch pots in the greenhouse. Half of these were heavily watered, the remainder only sparingly. Part of each group was fertilized at regular intervals to stimulate growth and the remainder left as controls. Ten days after blackheart began to develop, a large percentage of the heavily watered plants were affected, though no disease was visible in the sparingly watered ones.

TABLE I

THE EFFECT OF WATERING AND FERTILIZING ON THE DEVELOPMENT OF BLACKHEART

—	Heavily watered plants		Lightly watered plants	
	Diseased, %	Recovered, %	Diseased, %	Recovered, %
Plants fertilized	92.5	5.0	10.0	5.0
Plants not fertilized	100.0	80.0	10.0	10.0

It will be noted in Table I that the majority of the heavily watered plants became diseased, and little recovery occurred except in the controls, where growth was latterly retarded by lack of nutriment. In the lightly watered group, however, little blackheart developed and most of the diseased plants soon recovered. This experiment points out that normal, vigorous growth, rather than soil moisture, is the important factor, since in the heavily watered controls blackheart was arrested with the retardation in growth.

Atmospheric Humidity

In order to determine the effect of abnormally high humidity on the incidence of blackheart, plants growing in pots in the greenhouse were heavily and sparingly watered, and loosely enclosed in waxed paper cylinders which extended six or seven inches above the surface of the soil. The results as shown in Table II reveal that while heavy watering increased the disease to a considerable extent, enclosing the plants to increase the humidity in their immediate vicinity was a more significant factor.

TABLE II

THE INFLUENCE OF INCREASED HUMIDITY ON THE INCIDENCE OF BLACKHEART IN CELERY PLANTS

No. of plants	Percentage of diseased plants			
	Enclosed		Not enclosed	
	Heavily watered	Lightly watered	Heavily watered	Lightly watered
85	89	56	39	6
57	86	21	29	0

The Effect of Fertilizers

In four experiments involving 470 potted plants fertilized with varying proportions of nitrate of soda, superphosphate and muriate of potash, no significant variations in the incidence of blackheart were observed.

In an additional experiment the same chemicals were used, singly and mixed in various proportions, and quantities of each of the fertilizers containing an equal amount of soluble salts were added to separate groups of plants at weekly intervals for a period of seven weeks. In Table III it is shown that the majority of the plants which were growing

TABLE III

THE EFFECT OF FERTILIZERS ON THE DEVELOPMENT OF BLACKHEART

Fertilizer	Percentage blackheart	Plant growth*	Plant color
N	20	2	Dark green
P	80	5	Light green
K	40	2	Yellow
4-8-4	90	5	Green
4-4-8	100	5	Green
4-8-8	90	5	Green
4-4-12	90	5	Green
None	100	4	Green

* *The numbers 2, 4 and 5 indicate relative growth, 5 representing normal.*

normally became diseased, a further indication that the vigorous condition of the plant, and not the specific agent responsible for growth, is the important factor in the development of blackheart.

In a field test, two similar series of four plots were planted, one on a light sandy soil, the other on a heavy clay loam. The first plot in each series received manure, the second manure plus chemical fertilizer, the third fertilizer only, while the fourth was left untreated. The percentages of blackheart, which varied in each plot in direct proportion to the vigor of the plants, are shown in Fig. 1.

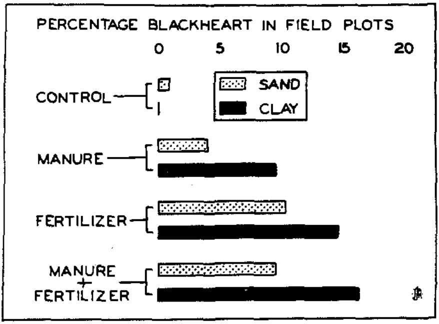


FIG. 1. *The effect of manure and fertilizer on the incidence of blackheart in field plots.*

Seeding and Planting Dates

Since field observations had established that blackheart was more severe in the early celery crops, and did not appear to any appreciable extent until the plants were nearing maturity, experiments were conducted for two seasons to determine the effect of seeding and planting dates on the incidence of the disease.

Samples of the same celery seed were sown on different dates and as the seedlings developed their second leaves they were transplanted into flats and kept under similar conditions. Later, these were planted in the field in three groups of plots, the first in the middle of May, the second early in June, and the third in the latter part of June. With the exception of the seedlings from the sowing of March 28, which were somewhat smaller than the others at the time of the earliest planting, there was little difference in the size and appearance of the plants when they were set, regardless of the date of seeding. This condition, however, soon disappeared and throughout the growing season there was little variation in growth within the groups planted on the same day, but those planted on the later dates were slightly smaller in each case than the ones planted previously.

An examination of the graph in Fig. 2 shows that the greatest amount of disease appeared in the earliest-set plants and there was little difference in any except those from the latest seeding, showing that under the same environmental conditions the date of planting had a greater influence on the incidence of blackheart than did the age of the plants.

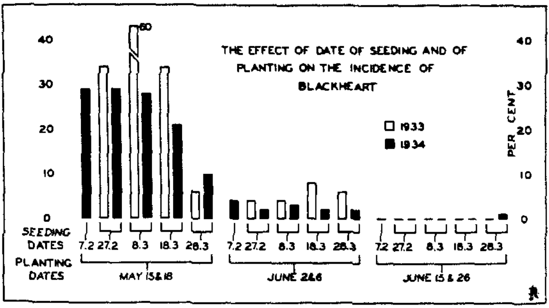


FIG. 2. *The effect of date of seeding, and of planting in the field, on the incidence of blackheart.*

This seems to indicate that within limits the susceptible stage for blackheart development in celery is governed by its growth in the field and, therefore, if planting is slightly delayed, the susceptible period does not coincide with the optimum causative environmental conditions, with the result that plants may escape the disease.

Varietal Susceptibility

To determine the existence of varietal susceptibility to blackheart, tests were conducted in 1936 and 1937 with 30 different samples of seed obtained from various sources and comprising white, green and pink varieties. One hundred and fifty plants of each were used each year. These were critically examined several times after the initial appearance of the disease. In 1936, owing to the abnormally dry conditions which prevailed, growth was slow and blackheart was not general, only a few of the varieties being affected. In 1937, however, with good growing conditions and a higher percentage of disease, more accurate determinations on varietal susceptibility were possible.

As has been previously stated, the amount of blackheart may vary from time to time in a given plot, some affected plants recovering after the initial attack and others continuing to exhibit symptoms. Each value in Fig. 3 represents the highest percentage of affected plants recorded and not the total amount of disease appearing throughout the season.

Although none of the varieties tested showed complete resistance, there were wide variations in reaction of commonly grown varieties. For example.

Golden Plume and Golden Phenomenal were quite resistant, while Paris Golden was highly susceptible.

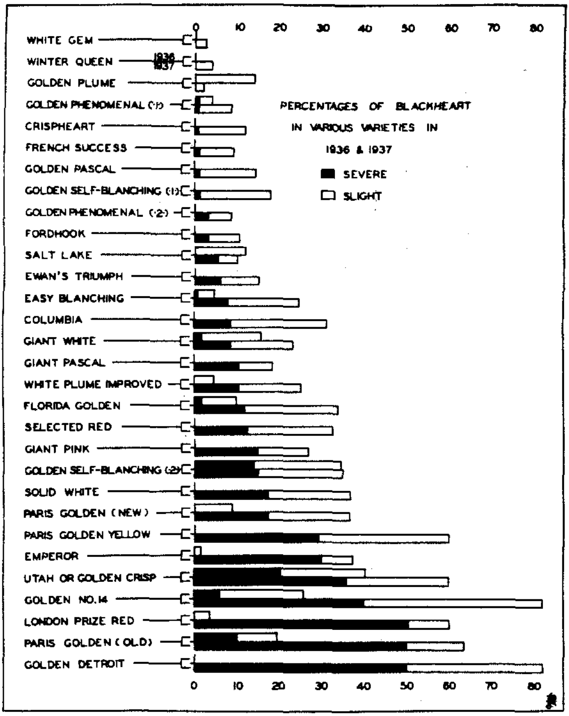


FIG. 3. *Varietal susceptibility to blackheart shown by the percentages of disease under field conditions in 1936 and 1937.*

Temperature and Humidity Tests

A systematic study of meteorological data established the fact that in the field blackheart developed after periods of high temperature or high humidity, or both. In order to determine the specific effect of these factors, potted plants both from the greenhouse and from outdoors were subjected to various atmospheric conditions by placing them for different lengths of time in a

cabinet in which temperature and light could be varied and relative humidity maintained in the higher ranges.

More than 350 plants were used in these tests, and as a check against the possibility that the mere removal of the plants from their original environment might induce the disease, an equal number of similar plants were placed in the immediate proximity of the cabinet.

Owing to the large number of experiments that were performed and the complexity of the variations tested, the results in Table IV are grouped to emphasize the salient features.

Plants in all stages of development were subjected to the tests, and their reactions varied both with the maturity of the plants and in groups of plants at similar stages of maturity. The results showed that temperatures above 85° F. combined with a relative humidity above 90% either in the presence or absence of light, were conducive to the development of blackheart, providing the plants were approaching maturity and in a susceptible condition. If, however, they were immature or exhibited a hard type of growth they seldom developed the disease.

TABLE IV

THE EFFECT OF TEMPERATURE AND HUMIDITY ON THE INCIDENCE OF BLACKHEART

Temperature, °F.	Relative humidity, %	Blackheart, %
85–95	90–100	85
65–80	90–100	28
90–93	85–90	37
75–85	10–50	30

Isolations from Affected Tissue and Examination of Roots

Isolations were made from plants showing typical symptoms of blackheart, to discover whether pathogenic organisms were associated with the necrotic tissues. The majority of plantings from 414 specimens obtained from 12 different sources revealed the presence of bacteria. Most of the isolates, however, were definitely saprophytic, a few were capable of producing slight rot on injured celery stalks *in vitro*, while two that resembled *E. carotovora* were definitely pathogenic.

Root systems of apparently healthy and typically diseased plants were also critically examined, both macroscopically and microscopically, to see whether symptoms of the disease were exhibited below ground, but no consistent differences could be observed.

Soft-rot

There is ample evidence of the existence of a soft-rot of celery caused by *Erwinia carotovora* (L. R. Jones) Holland (10, 11, 17, 18). The disease has been thoroughly investigated and is only briefly considered here in its connection with the blackheart problem since under field conditions a secondary bacterial soft-rot is frequently associated with the physiological disease.

Symptoms and Cause of the Disease

Soft-rot of celery manifests itself as a soft, watery, light-brown decay, which, under moist conditions rapidly destroys the affected part of the plant. If the

heart becomes involved, the plant is generally destroyed. Isolations from typically rotted plants revealed the presence of short, flagellate, rod-shaped bacteria, resembling *E. carotovora* (L. R. Jones) Holland.

Experiments

Inoculations

A large number of inoculations made on plants, both in the greenhouse and under field conditions, proved that the pathogenic bacteria were unable to infect uninjured tissues, but, if abrasions were present on any part of the plant and humid atmospheric conditions prevailed, they could produce infection within a few hours, and totally destroy the affected part in a week or ten days.

These experiments demonstrated that humid atmospheric conditions were necessary for both initial infection and disease development. Although its progress was often inhibited in a dry environment, the pathogen remained viable for a considerable time, and upon the recurrence of favorable conditions was capable of resuming its activity. If the inhibition occurred after the heart leaflets had been killed and before the infection had progressed far down the petiole, the diseased tissues presented an appearance somewhat resembling physiological blackheart, though the necrotic tissues were typically brown instead of black and the leaflets somewhat less shrivelled.

Secondary Decay

When blackheart is severe, affected plants frequently exhibit a definite "soft-rot" of the heart tissues. Although *E. carotovora* was isolated from several such plants, the flora in the affected tissues was usually so varied that the species responsible for the condition could not be determined. Therefore a number of experiments were conducted to ascertain the possible role of the soft-rot bacteria in this connection.

Celery plants affected with blackheart were sprayed with water suspensions of *E. carotovora* and kept under humid atmospheric conditions, since it had previously been shown that in a dry environment the pathogen was inactive. Of the 30 plants inoculated as described, all developed soft-rot in the necrotic blackheart-affected tissues. Of a like number of control plants kept under similar conditions but sprayed with sterile water, only 6.6% developed soft-rot from which bacteria similar to *E. carotovora* were isolated. These results clearly show that plants affected with blackheart may be destroyed by a secondary bacterial heart-rot, since in the field *E. carotovora* may be present on the plants, ready to become active when proper environmental conditions occur.

The Relation of *Lygus pratensis* L. to Blackheart and Soft-rot

The insect *L. pratensis* has a definite bearing on the present problem. It is a common pest on celery, particularly in the early part of the season, and the injuries caused by its feeding activities have often been confused with physiological blackheart. In addition, when soft-rot is present, it is an important factor in the spread of this disease.

Although tarnished plant bugs are frequently present in large numbers, particularly on early celery, their relation to blackheart could not be determined from field observations, since severe cases of the disease were observed in both the presence and absence of the insects. The possibility that they might feed on the young heart tissues and produce a necrotic condition resembling blackheart led to a series of experiments conducted to determine the type of injury produced.

Experiments

Nature of the Insect Injury

It was found that in addition to the direct injury resulting from their feeding habits, tarnished plant bugs also produced a toxic effect on the host, since microscopic examination of affected tissues invariably showed necrotic cells beyond the zone of ruptured tissue. Numerous punctures in a restricted area severely injure the vascular tissue, resulting in a chlorosis or death of the leaves beyond the affected point.

The epidermal cells are usually not severely damaged, the necrosis being confined largely to the underlying parenchymatous and vascular tissues. This results in irregularly shaped, ill-defined lesions of a dull grayish-brown color, which varies in intensity with the extent of the injury.

In Ontario early celery crops are more severely affected by these insects than those planted later, and since physiological blackheart also developed on the early crops it was impossible to determine the amount of damage directly due to the insect feeding.

At a season when blackheart seldom occurred, insect-free plants were protected under cloth cages both in the field and in the greenhouse. Large numbers of tarnished plant bugs were liberated in half of these cages, and the remainder served as controls. Within the next few days, discolored areas appeared on many of the petioles of plants in the cages containing the insects.

After the plants had been subjected to insect attack for three weeks under field conditions, and a somewhat shorter period indoors, they were all critically examined. The plants protected from insects presented a somewhat chlorotic and etiolated appearance, due to shading, but bore no withered leaves or necrotic lesions of any kind. On the other hand, the plants subjected to insect attack showed severe damage to many leaves, but the results of feeding were evident only on the fleshy stalks and leaf petioles, particularly at their junction. The older leaves were invariably more severely affected than the younger ones, and in many cases the vascular tissue was destroyed, death of the entire leaf being the result. No condition even remotely resembling blackheart was observed, since when the young leaves were affected, the damage was only apparent on the petioles and leaf stalks, not on the small succulent leaf blades.

Insects and Bacteria

The role of the insect *L. pratensis* L. in the dissemination of soft-rot is extremely important. In each of the many experiments conducted in this connection, these insects were allowed to feed upon growing plants which had

been sprayed with a water suspension of *E. carotovora*. In the tests conducted under moist environmental conditions, bacterial infections developed in the feeding punctures within 48 hours after the insects were placed on the plants, and spread rapidly until the infected leaves were destroyed, but, as was the case with all other bacterial inoculations, no infection occurred when the humidity was low. As controls, insects were allowed to feed on plants which had been sprayed with sterile water, and plants were sprayed with bacteria in the absence of insects. In a few cases in the latter group, infections occurred but their origin could always be traced to abrasions of the host tissues.

Discussion

Although physiological blackheart, bacterial soft-rot and insect injury each produce distinct and characteristic symptoms readily recognizable in their early stages, their combined effects were frequently so complicated that it was often difficult and sometimes impossible to determine the initial cause of the trouble in the field. As a result of these investigations it is possible by careful observation to determine which of the factors in question is responsible for the damage.

The necrosis associated with blackheart always originates in the small heart leaflets or folded leaves, and develops into a black, leathery dry rot, while the insects in question confine their feeding mainly to the fleshy petioles and damage the leaves by interfering with the function of the vascular tissues. On the other hand, infections by the wound parasite *E. carotovora* may originate on any part of the plant, through necrotic tissue, insect punctures or abrasions of any kind, and cause a typical light brown, moist soft-rot.

During the critical investigations with blackheart several significant facts presented themselves. A study of meteorological data revealed that in the field the disease appeared during or after periods of high temperature or high humidity, or both. In a controlled environment it was found that both conditions were necessary, since when either was lowered, considerably less disease resulted. Furthermore, the disease did not develop to any appreciable extent until the plants were nearing maturity. The maturity factor was evident in both greenhouse and outdoor plants, but whereas it was only the early planted crops which developed the disease in the field, the predisposing atmospheric conditions were not dependent on the season under greenhouse conditions. The maturity factor as related to a plant's susceptibility to blackheart is, within limits, independent of its age, but is governed by the date of planting in the field, since results showed that there was little variation in the amount of disease in plants set on a given date regardless of their ages. It is only when the plants are set early that they reach the susceptible stage coincident with the occurrence of predisposing factors.

In addition, it was noted that the disease was most severe in vigorous plants, regardless of the factor responsible for the vigor, and that definite differences in susceptibility to the disease are exhibited by different varieties. Blackheart of celery can be described as the death of the heart tissues due to

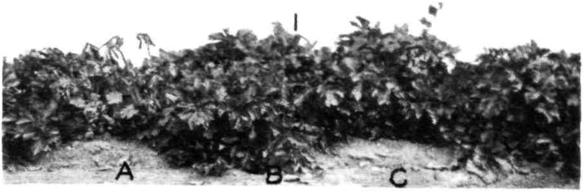
PLATE I

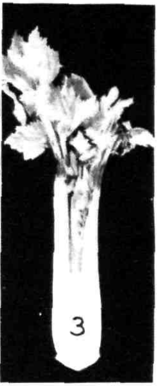
FIGS. 1 AND 2. *Black heart on young celery leaves from specimen growing in field.* FIG. 3. *Primary symptoms of heart leaf on a plant growing in the greenhouse.* FIG. 4. *A typical specimen of black heart obtained from the field.* FIG. 5. *Black heart produced in the greenhouse.*

2

3

FIG. 1. *Initial infections caused by needle prick inoculations with soft-rot-producing bacteria shown at arrows. One week later these plants were entirely rotted.* FIGS. 2 AND 3. *Plants similarly affected with black heart after being kept for one week in a humid environment. (2) was sprayed with soft-rot-producing bacteria and shows the rotted heart leaves due to infection which entered through the necrotic tissues. The plant in (3) remained unchanged demonstrating the non-parasitic nature of black heart.*





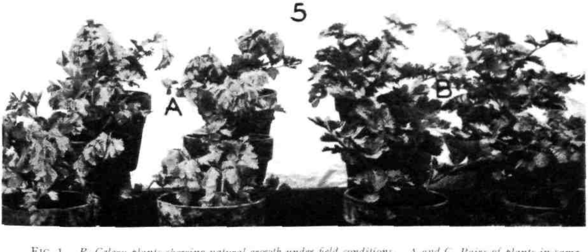


FIG. 1. *B, Celery plants showing natural growth under field conditions. A and C, Pairs of plants in same row as B, covered by insect-proof cotton cages. A, containing tarnished plant bugs and C, not.* FIG. 2. *Plant fed upon by Lygus pratensis L. Note damage caused by feeding punctures at juncture of leaf blades and petioles.* FIG. 3. *Head of celery showing damage on the larger petioles caused in the field by Lygus pratensis L.* FIG 4. *A duplicate of (2) except that the plant was sprayed with soft-rot-producing bacteria before being fed upon by the insects.* FIG. 5. *A, Six plants caged under indoor conditions and fed upon by Lygus pratensis L. Note the chlorotic and flaccid condition of the older leaves due to the heavy feeding on the upper parts of the petioles. B, Six control plants showing the normal type of growth.*

the advent of unfavorable environmental conditions at a critical stage in the development of the plant.

In connection with the studies on soft-rot it was found that in the districts under consideration *E. carotovora* (L. R. Jones) Holland, was of importance only in that, under humid environmental conditions, the bacteria could cause a secondary decay following blackheart or insect injury.

Acknowledgments

Sincere thanks and appreciation are extended to Dr. G. H. Berkeley for the many helpful suggestions and invaluable criticisms given throughout the progress of this investigation; to the Dominion Horticulturist for supplying samples of celery seed; and to Mr. W. A. Ross of Vineland for suggestions in connection with the insect phase of the paper.

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ORGANIC MERCURY FUNGICIDES AND DISEASE RESISTANCE IN THE CONTROL OF SLENDER WHEAT GRASS SMUT[1]

By A. W. Henry[2], S. B. Clay[3], and J. R. Fryer[4]

Abstract

The common smut disease of slender wheat grass, *Agropyron pauciflorum*, was completely controlled by treatment of naturally or artificially smutted seed with three organic mercury dust fungicides containing as active ingredients ethyl mercury phosphate, methyl mercury nitrate and methyl mercury phosphate, respectively. Applications of one-half ounce per bushel gave as satisfactory control as higher rates and caused no appreciable seed injury after storage of the treated seed for one year.

Several Alberta collections of wild plants of *Agropyron pauciflorum* and of intermediates between *Agropyron pauciflorum* and *Agropyron subsecundum* proved highly resistant or immune from smut when artificially inoculated at Edmonton, while other collections proved moderately or highly susceptible.

Fyra, a superior variety of slender wheat grass which has been distributed for several years by the University of Alberta, has been shown to be highly smut resistant but not immune.

As long as smut-susceptible strains of slender wheat grass are grown, and until immune varieties are developed and generally distributed, continuance of seed treatment is advised.

Introduction

The common smut of slender wheat grass, *Agropyron pauciflorum* (Schwein.) Hitchc., is the chief smut disease affecting a cultivated forage grass in western Canada. This disease was first reported from Saskatchewan, and the causal fungus was identified as *Ustilago bromivora* (Tul.) Fisch. v. Waldh (4). More recently Fischer has proposed that this smut fungus be included in the composite species *Ustilago bullata* Berkeley (1). Owing largely to the fact that it is seed borne, this smut has become widely distributed in the central part of the North American continent, and has assumed considerable economic importance especially in crops intended for seed purposes. Anyone who contemplates growing slender wheat grass is consequently well advised to take precautions against the disease.

Up to the present time the principal control measure suggested has been seed treatment with formaldehyde. This method was first found effective in the prevention of this smut by Fraser and co-workers (2, 3, 4). Their treatment consisted in soaking the seed for five minutes in a 1–320 (one part commercial formaldehyde to 320 parts of water) solution, covering for two hours

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[2] *Associate Professor of Plant Pathology, University of Alberta.*
[3] *Graduate Assistant in Plant Pathology, University of Alberta.*
[4] *Professor of Genetics and Plant Breeding, University of Alberta.*

and then spreading out to dry. Tests made over a period of several years from 1918 to 1926 not only gave excellent control of the smut, but also showed no evidence of seed injury.

Fraser and Scott (4) also experimented with a dry fungicide, namely, copper carbonate dust, but failed to control the smut satisfactorily.

The possibility of preventing this smut by regulation of the time of seeding was investigated by Padwick and Henry (6), but it was concluded by them that this method held little promise of success.

The use of smut-resistant or immune varieties or strains of slender wheat grass would obviously be a desirable preventive measure, as it has proved to be in various other crops. As far as we are aware, however, the possibility of control by this method has not been previously explored and no reports of smut resistance in this grass have come to our attention.

Objects of Present Investigations

The studies reported here were undertaken with two objectives in view, namely, finding (i) a fungicide which would successfully control this smut disease when used for seed treatment in a dry or dust form, and (ii) resistant or immune strains of the grass host which might be used to replace susceptible varieties or strains, or to serve as parent material for breeding work.

Seed Treatment Studies

Although, as has been pointed out, Fraser and Scott (4) have obtained excellent control of slender wheat grass smut by treating infested seed with a 1–320 formaldehyde solution, this treatment has several obvious disadvantages. Compared with a dry treatment it is less convenient, more time consuming and less suitable for use in advance of seeding time. Moreover, there is danger of seed injury, especially in the hands of careless users who may apply overdoses. While Fraser and Scott (4) report no evidence of seed injury, and we have had relatively little, Morwood (5) found that formalin adversely affected germination when applied to the seed of *Bromus unioloides*.

The only dry treatment heretofore reported to our knowledge for the control of slender wheat grass smut is the copper carbonate dust treatment. Fraser and Scott (4) used copper carbonate, but failed to obtain satisfactory smut control with it. Other dusts, however, have been used for the treatment of grass seed. The work of Morwood (5) on *Bromus unioloides* should again be noted in this connection, since he used two organic mercury dusts, Abavit B and Ceresan, for the control of a smut disease similar to that affecting slender wheat grass. The applications which he used were heavy, namely, three ounces per 20 pounds of seed. These reduced infection from 83.5% in the controls to 0.1%.

Experimental Methods

Investigations on the control of slender wheat grass smut by seed treatment with fungicidal dusts were begun at the University of Alberta in 1931, but no conclusive data were obtained until 1936. In the early experiments* little

* *The assistance of Messrs. G. W. Padwick and J. B. Skaptason in connection with these experiments is gratefully acknowledged.*

or no smut developed in the untreated checks, so no conclusions could be drawn from them. These failures were at first attributed to unfavorable soil conditions, but in the light of the studies of Padwick and Henry (6) and of those reported in the latter part of this paper, they now appear to be due to the use of a smut-resistant variety of slender wheat grass.

In 1936 extra precautions were taken to insure smut infection in the checks. To this end a composite sample of seed was used for the main experiment. This consisted of three commercial lots each obtained from a different local seed store. This seed mixture was then dusted with a composite collection of smut spores obtained from five separate Alberta sources. Three were obtained from smutty stands of slender wheat grass growing in the vicinity of Edmonton, one from Strathmore in southern Alberta, and one from Vermilion in the east central part of the province. In another experiment naturally smutted seed was used, as it was thought possible that it might respond differently to seed treatment than would artificially smutted seed. The naturally smutted seed was obtained in 1936 from plots at the University that contained a considerable proportion of smutted plants.

The fungicidal values of five chemicals, namely, formaldehyde, copper carbonate, ethyl mercury phosphate, methyl mercury nitrate and methyl mercury phosphate were tested on the artificially smutted seed, but only the last three were tried on the naturally smutted seed.

The formaldehyde treatment, the only wet treatment tested, consisted in these experiments of a two-minute soak in a solution containing one part of commercial formaldehyde and 320 parts of water. The treated seed was then covered with sacks for four hours and finally air-dried before seeding.

The dry chemicals or dusts were applied simply by shaking a weighed amount of each in a flask with 25 grams of seed. A copper carbonate dust containing 50% copper carbonate was applied at the rate of two ounces per bushel of seed and three organic mercury dusts, namely, ethyl mercury phosphate*, methyl mercury nitrate**, and methyl mercury phosphate†, with mercury equivalents of 3.8, 1.5 and 3% respectively, were each applied at three different rates, namely, one-half, one and two ounces per bushel of seed.

After treatment the seeds were counted and later sown in the field at the rate of 200 seeds per rod row. Four replications of each treatment were sown. In spite of being sown late in the summer, namely, on August 3, 1936, the stands survived the winter well and headed normally the following summer.

The smut data were taken by counting the number of heads per row and recording the smutted heads as a percentage of the total number.

Smut Control in Field Experiments

The field results from the seed treatment experiments are given in Tables I and II. The smut data are averages of four replications in each case. It will be noted that two counts are given for each treatment. These represent

* *Sold under the trade name of New Improved Ceresan.*

** *Sold under the trade name of Leytosan.*

† *Sold under the trade name of Leytosan P.*

results from two crops in 1937. After the first one had headed and was counted, a second one was allowed to head and a separate count was made on it. Table I gives the results obtained for plants from seed which was both naturally and artificially smutted.

TABLE I

RELATIVE EFFECTIVENESS IN SMUT PREVENTION OF DIFFERENT FUNGICIDES USED FOR SEED TREATMENT OF BOTH NATURALLY AND ARTIFICIALLY SMUTTED SEED OF SLENDER WHEAT GRASS

Treatment	Amount of fungicide per bushel, oz.	Percentage of heads smutted		
		First crop	Second crop	Average
None—check (naturally smutted)	0	5.5	7.8	6.6
None—check (naturally and artificially smutted)	0	14.4	16.8	15.6
Formaldehyde 1–320	–	0.0	0.0	0.0
Copper carbonate	2	5.4	4.2	4.8
Ethyl mercury phosphate	½	0.0	0.0	0.0
Ethyl mercury phosphate	1	0.0	0.0	0.0
Ethyl mercury phosphate	2	0.0	0.0	0.0
Methyl mercury nitrate	½	0.0	0.0	0.0
Methyl mercury nitrate	1	0.0	0.0	0.0
Methyl mercury nitrate	2	0.0	0.0	0.0
Methyl mercury phosphate	½	0.0	0.0	0.0
Methyl mercury phosphate	1	0.0	0.0	0.0
Methyl mercury phosphate	2	0.0	0.0	0.0

It will be seen from the data in Table I that the formaldehyde treatment and all the mercury dust treatments completely controlled the smut, whereas copper carbonate dust treatment did not. In the case of the mercury dusts it is noteworthy that the one-half ounce rate in each case was as effective as heavier applications.

The mercury dusts were tested further on another sample of seed which was naturally smutted only. In this case only one rate of application, namely one ounce per bushel, was used. The results are given in Table II.

The effectiveness of the mercury dusts in controlling this smut is again shown in Table II. When used at the rate of one ounce per bushel all three dusts tested gave perfect control. While they have given no better control

TABLE II

RELATIVE EFFECTIVENESS IN SMUT PREVENTION OF DIFFERENT ORGANIC MERCURY DUSTS USED FOR SEED TREATMENT OF NATURALLY SMUTTED SEED OF SLENDER WHEAT GRASS

Treatment	Amount of fungicide per bushel, oz.	Percentage of heads smutted		
		First crop	Second crop	Average
None—naturally smutted	–	9.4	10.0	9.7
Ethyl mercury phospate	1	0.0	0.0	0.0
Methyl mercury nitrate	1	0.0	0.0	0.0
Methyl mercury phosphate	1	0.0	0.0	0.0

than formaldehyde, they have some decided advantages over this treatment, as has already been indicated, and over copper carbonate because of its failure to give complete control. It is true that the percentage infection in the checks in the above experiments is not especially high, but the control is consistent in all tests and would seem adequate for practical purposes.

Effect of Treatments on Seed Viability

The field plots were not sown in such a way as to make possible an accurate estimation of the effects of the treatments on the germinability of the seed, but an examination of the stands in the seedling stage revealed no obvious differences between the treatments or between the checks and the treatments.

Samples of the treated and untreated seed used in the field experiments were, however, saved. These were stored for a year in the laboratory at room temperatures in small stoppered 200 cc. Erlenmeyer glass flasks. Representative samples of this stored seed were then tested for viability in flats of soil in the greenhouse. The results of these tests are given in Table III.

TABLE III

VIABILITY OF TREATED AND UNTREATED SEED OF SLENDER WHEAT GRASS AFTER STORAGE FOR ONE YEAR IN STOPPERED FLASKS

Treatment	Amount of fungicide per bushel, oz.	Per cent emergence				
		A*	B	C	D	Average
None—check	–	92	96	80	98	92
Formaldehyde 1–320†	–	100	76	88	92	89
Copper carbonate	2	92	80	96	100	92
Ethyl mercury phosphate	½	88	92	88	92	90
Methyl mercury nitrate	½	96	90	88	88	90
Methyl mercury phosphate	½	90	88	92	94	91
Methyl mercury phosphate	2	62	64	64	64	64

* *A, B, C and D represent four replicates. The value for each replicate is based on 50 seeds tested, except in the cases of formaldehyde and copper carbonate tests where it is based on 25 seeds per replicate.*

† *The formaldehyde treated seed was not stored. The treatment was applied in this case just previous to seeding time.*

It is evident from Table III that none of the treatments covered in the seed viability tests caused appreciable decreases in emergence except methyl mercury phosphate at the rate of two ounces per bushel. Since the tests were made on seed which had been stored for a year they would seem more likely to disclose any evidences of seed injury than tests made immediately or shortly after treatment. It should be noted especially that the mercury dusts at one-half ounce per bushel caused no significant reduction in emergence nor did formaldehyde applied immediately before seeding and, as will be remembered, all these treatments controlled the smut disease completely. Copper carbonate caused no seed injury, but, as has been noted, it failed to control the smut satisfactorily. While the two-ounce rate of mercury phosphate

caused serious injury, it is unnecessary to use such a high rate of application for the control of the smut disease in question. Injury in this case was further evident in a noticeably lowered vigor of the seedlings as compared with the checks and the other treatments.

Smut Resistance in Slender Wheat Grass

Smut Reaction of Wild Plants

Slender wheat grass, *Agropyron pauciflorum*, and several other species of *Agropyron* are native to this part of the North American continent. In Alberta, *Agropyron pauciflorum* is commonly found in the wild state in the central, western and northern parts of the province. Another closely related species, *Agropyron subsecundum* (Link) Hutch., bearded wheat grass, occurs abundantly in the same habitat, and types intermediate between the two, which are apparently natural hybrids, are frequently found. Although by artificial inoculation *Agropyron pauciflorum* has been found highly susceptible and *Agropyron subsecundum* moderately susceptible to the smut fungus from *Agropyron pauciflorum* (4, 6), wild plants of these species are very rarely observed to be smutted. It appeared possible therefore that there might be smut-resistant individuals among some of these wild plants, and if so, they might be used in the development of smut-resistant strains or varieties.

Consequently, opportunities being provided during the course of Plant Disease Survey trips in 1932–33, collections of seed of wild plants were made from different parts of Alberta. In most cases the collections were from plants typical of *Agropyron pauciflorum*, but in some instances as judged from the progenies of the plants, intermediate types between *Agropyron pauciflorum* and *Agropyron subsecundum* apparently were chosen.

Seed of the above collections of wild plants was artificially smutted with smut obtained from diseased stands of *Agropyron pauciflorum* growing at Edmonton. Each collection was assigned a number and sown separately in rows in the field at Edmonton in the summer of 1935. Most of the collections survived the winter and headed in 1936, when notes were taken

TABLE IV

Smut reaction of progenies of wild plants of *Agropyron pauciflorum* and intermediates between *Agropyron pauciflorum* and *Agropyron subsecundum*

Number of strain	Place collected	Per cent of smutted heads
I-35-4	Mundare	0.0
I-35-5	Bentley	16.0
I-35-8	Westlock	14.0
I-35-13	Vermilion	33.0
I-35-14	Hughenden	0.0
I-35-15	Lloydminster	0.0
I-35-16	Chauvin	0.0
I-35-17	Provost	0.0
I-35-18	Sedgewick	6.0
I-35-21	Lloydminster	1.5
I-35-22	Chauvin	21.0
I-35-1	Tofield	26.0
I-35-3	Colinton	50.0
I-35-6	Sylvan Lake	36.0
I-35-7	Edmonton	8.0
I-35-9	Barrhead	1.0
I-35-10	Legal	30.0
I-35-11	Fort Saskatchewan	0.0
I-35-19	Edmonton	0.0
I-35-20	Daysland	12.0
I-35-23	Ranfurly	0.0

on the percentage of smutted heads. The total number of heads in each of the progenies was counted and the number of smutted heads was recorded as a percentage of the total. The data on smut reaction of these are given in Table IV. Approximately one-half of them (listed first in the table above the horizontal line) proved quite typical of *Agropyron pauciflorum*, producing slender, awnless, bilateral heads. There were, of course, some variations in color, height, leafiness, habit of growth and other characters. The remainder appeared intermediate between *Agropyron pauciflorum* and *Agropyron subsecundum*. The majority of these intermediate types produced unilateral heads with short awns, and rather broad, dark green leaves.

It is evident that there are wide differences in smut reaction among the progenies of the wild plants listed in Table IV. Some are quite susceptible, but a considerable proportion appear to be immune or highly resistant to the collection of smut with which they were inoculated. Those which developed no smut in these tests are not necessarily to be considered immune to all strains of smut from *Agropyron pauciflorum*, but subsequent tests have indicated that most of them are highly resistant or immune to other collections of this smut, and hence might serve as parental material for breeding work in the development of resistant or immune varieties.

Smut Resistance of the Variety Fyra

In the first part of this paper, which dealt with seed treatment, mention was made of the fact that the first tests on the relative effectiveness of different fungicides in smut prevention yielded no results, owing to the failure of the checks to develop any smut. In these tests the variety Fyra, an improved variety of slender wheat grass developed at the University of Alberta, was used. No data on the smut reaction of this variety had previously been obtained. The seed treatment tests, however, indicated that it might be resistant, since the tests were made in three different years with the untreated checks replicated each year, and no smut developed in them. We have now tested this variety along with commercial nondescript lots of seed, with several collections of smut, and determined that it is highly resistant to, but not immune from smut. Stands from the commercial seed under the same conditions were badly smutted. It is therefore now possible to state that the variety Fyra, in addition to its other desirable qualities, possesses high smut resistance though not general immunity.

Discussion

The results of the studies here reported show that the common smut of slender wheat grass may be prevented by seed treatment with organic mercury dusts and by the use of disease-resistant strains of the grass.

The organic mercury dusts gave complete control at rates as low as one-half ounce per bushel, whereas copper carbonate dust at two ounces per bushel, failed to control the smut satisfactorily. The organic mercury dusts have certain advantages over the wet formaldehyde treatment which has

been recommended for the control of this smut, though the latter is effective in smut prevention. Treating several months ahead of seeding, for instance, would seem safe with the organic mercury dusts tested, if applied at the one-half ounce rate, since no appreciable reduction in viability was noted after storage of treated seed for one year.

The occurrence of high smut resistance or immunity among local wild plants of *Agropyron pauciflorum* and among types intermediate between *Agropyron pauciflorum* and *Agropyron subsecundum* is reported apparently for the first time. These resistant plants obviously could be used in combating this smut in another way, namely, by using them in the breeding of resistant varieties. The studies, however, also bring to light the fact that at least one improved smut-resistant variety already exists, namely, the variety Fyra, pure seed of which has been distributed for several years by the University of Alberta. It may, however, be possible to improve upon the resistance of this variety which, as has been noted, is not immune, by re-selection or by crossing it or other superior varieties with highly resistant or immune wild selections.

While the use of immune varieties may eventually do away with the necessity of seed treatment, it would seem advisable at present to continue treatment as an insurance against smut, even in varieties like Fyra. This is particularly important in crops grown for seed and especially so in a perennial crop like slender wheat grass, since a stand once infected remains so throughout its life.

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STUDIES ON *RHIZOCTONIA SOLANI* KÜHN.

IV. EFFECT OF SOIL TEMPERATURE AND MOISTURE ON VIRULENCE[1]

By G. B. Sanford[2]

Abstract

The effects of soil temperatures between 16° and 25° C., and of soil moisture content between 19 and 40% of the moisture-holding capacity, on the virulence and type of attack of *Rhizoctonia Solani* on young potato sprouts, were studied under controlled conditions and the results from 13 separate tests are discussed. The comparative growth rates of the pathogen on nutrient agar and in soil are outlined.

At 25° C. the disease diminished very abruptly. Between 23° and 16° C., the pathogen appeared equally virulent throughout the range of soil moisture mentioned. The fluctuations which occurred in separate tests were not definite or consistent enough to warrant a conclusion that the virulence is greater at 16° than at 23°, or that a dry soil is more or less favorable to it than a wet one.

In a fertile, steam sterilized loam, at medium moisture content, it required about ten days for the pathogen to grow as far as it did on the surface of a nutrient medium in four days. The growth rate at either 23° or 16° C. was slightly higher in a wet soil than in one of medium moisture content, but in a dry soil the rate was somewhat less at 23° than at 16° in a medium or wet soil. Even in a fairly dry soil (19% moisture-holding capacity) at 16° the growth of the pathogen covered a distance of 5 cm. in ten days, which would appear adequate for infection of young sprouts from a set bearing viable sclerotia.

The effort of the host to recover, by means of secondary and tertiary sprouts from the attacked primary sprout, was better in a wet soil than in a dry one at both 16° and 23° C. The best effort was in a wet soil at 23°. A distinction is made between the effects of soil moisture and temperature in stimulating growth of the host, and their effect on parasitism itself.

The remarkable tendency of the secondary sprouts to escape infection, regardless of soil temperature and soil moisture, is indicated. There was evidence that certain factors other than soil temperature and moisture may play an important role in the parasitism of *R. Solani*.

In a previous paper (13) of this series, it was demonstrated that the development of stem canker of potato during the first 30 days in May was very slight in some fields, but rather severe in others. This occurred in the same locality, despite the fact that sets from the same source, uniformly and heavily infested with sclerotia, were used in all experiments. That some of this erratic behavior among experiments in various fields each year may have been influenced by differences in soil moisture seemed possible, because, in the many different crop sequences involved, this factor naturally would not be very uniform. Also, no doubt, the temperature varied slightly from field to field in the same district, but available records indicate that the temperature factor was fairly uniform in most experiments, and at least well within the range for vigorous disease development during the short duration of the test. On the other hand, cases occurred in which absence of disease could not be accounted for by differences in either soil moisture or soil temperature.

The relation of temperature to the development of disease on potatoes by *R. Solani* has been discussed by a number of workers, but the factor of soil

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Contribution No. 544 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, co-operating with the Department of Field Crops, University of Alberta.
[2] *Pathologist-in-Charge, Dominion Laboratory of Plant Pathology, Edmonton, Alberta.*

moisture does not appear to have received sufficient study. Therefore, it seemed advisable at this stage to investigate, under controlled conditions, the possible relation of both factors indicated.

Literature Review

The work of Müller (7), Newton (8), Le Clerg (4), Wellman (15), Monteith and Dahl (6) and others indicates that the optimum temperature for growth of the fungus *R. Solani* on nutrient agar may vary from approximately 23° to 30° C., depending on the strain involved, and to some extent on the host to which each was pathogenic. The optimum for strains known to be pathogenic to the potato usually varied between 24° and 27°, with 25° perhaps the most common.

With regard to soil temperature, the conclusions of Richards (12) have special significance to the present study, and therefore may, with advantage, be reviewed more fully here. He obtained lesions on potato stems from 9° to 30° C., but observed that only at temperatures below 24° was *R. Solani* seriously parasitic. Injury to the cortex of young stems during the first six weeks was especially severe between 12° and 21°, but most severe at 18°. Greatest destruction of the growing tips of young sprouts occurred between 12° and 18°. But above 18° this type of injury became less and less, and disappeared entirely at about 21°. Apparently he thought that the more rapid growth of the shoots from 21° to 24° was an important factor in the escape of the growing tips from injury, but on the other hand he states: "The pathogenic power of the parasite in fact appeared to be so distinctly inhibited by soil temperatures above 21° as to render the fungus practically unimportant as a pathogen upon the potato above 24° C." These two phenomena, *viz.*, the growth of shoot and the pathogenic power of the fungus, will be discussed later.

Müller (7) obtained greater damage to the growing tips of the sprouts between 12° and 15° than from 18° to 21° C., and Gratz (3) reported no attack on potato stems between 22° and 25° C.

Definite experimental evidence on the effect of soil moisture is apparently very meagre. In the field experiments of Richards (12), in 1918 and 1919, the disease was more severe in the former year when the soil was dry and cool during early plantings than in 1919, when the soil was warmer and more moist. He apparently thought that the marked differences in disease observed could be explained satisfactorily on the basis of temperature. Balls (1), Müller (7) and Orton (9) were of the opinion that the disease was favored in an over-moist soil. According to Peyronel (11), a soil that fluctuates in moisture content between a dry and moist state, favors disease. Martin (5), from field observations, reported that greater damage to potatoes occurred in places where the soil was deficient in moisture than where it was normal. Peltier (10), in connection with stem rot of *Dianthus caryophyllus*, states, "The conditions under which all strains manifested their greatest parasitism were primarily a high temperature (above 88° F.) and a soil moisture content either too low or too high for best development of the plant."

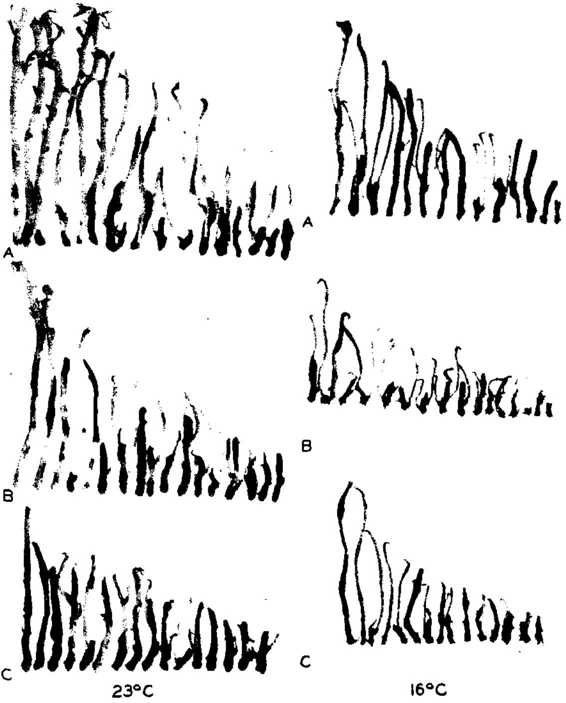

PLATE I. *The relative severity and type of attack produced on potato sprouts by Rhizoctonia Solani during 21 days, in relation to soil temperatures of 16° and 23° C., and soil moisture contents of (A), 38%; (B), 27%; and (C), 21%, moisture-holding capacity. Experiment 1, Table I.*

Materials and Methods

The method for determining the relative effect of soil temperature and soil moisture on the disease was very simple. Both factors were studied at the same time in parallel series. The only difference between the two series indicated was that of soil moisture and soil temperature. The general technique of preparing and infesting the soil was identical with that described in a previous paper (14). For instance, a natural, unsterilized, virgin prairie black loam was uniformly infested with the pathogen in the proportion of one part of inoculum to 15 parts of natural soil. This inoculum consisted of sterilized soil permeated with the pathogen. The containers were one-litre, wide mouth, Erlenmeyer flasks. A clean set (Early Ohio variety), of uniform size and surface, disinfected in acid mercuric chloride solution, was placed near the bottom of each flask and covered with the infested soil to a depth of five and one-half inches. The neck of each flask was then plugged with cotton. The sets were cut from tubers kept in cold storage at approximately 3° C.,* and were in the semi-dormant state when planted. This precaution was found to be very important in securing uniform results. There were 16 flasks, each containing one plant, in each unit of the experiment. Each test required 21 days from the date of planting. A numerical disease rating was given and other notes taken on each plant.

Soil Moisture

After the soil was infested, representative portions of it, sufficient for each test, were adjusted to dry, medium and wet soil moisture content (on the basis of the moisture-holding capacity of the soil) as required, and then put into the flasks. The percentage of soil moisture of each series varied only slightly from time to time when the experiment was repeated. Soil moisture determinations were made at the beginning and end of each test. These are indicated in Table I. The cotton plugs maintained the soil moisture to within approximately 1 to 3% of each initial value during the test, depending on the moisture content of the soil and the temperature. Thus, the fact that no further watering was necessary removed an important variable which exists when water is added to the soil from time to time.

Soil Temperature

The effect of temperatures from 16° to 23° was investigated because, as indicated in the literature review, there appears to be a difference of opinion regarding the influence of temperature within these limits on the degree and type of injury produced.

Results

Effect of Soil Moisture and Soil Temperature on the Disease

The results from 13 separate tests to determine the effect of soil moisture and soil temperature on the severity of the disease, are summarized in Table I. Two types of injury are described, *viz.*, "T" and "S". In the former, the primary sprouts are blighted soon after emergence from the sets and the

* *The Centigrade scale is used throughout this study.*

attack is severe. In the latter, the attack on the primary sprout is deferred and usually restricted to fairly small lateral lesions. The table shows which of these two types of injury prevailed, and also the percentage of plants which escaped entirely, because these data assist one to judge the relative severity of the disease. As a rule, the relation was close between a low percentage of plants that escaped and the "T" type of injury, and the severity of the attack. A disease rating of 50% or greater, combined with the "T" type of injury and few or no plants escaping, is certainly a very severe attack, and one which, on a different basis of estimate, might be as well expressed by 70% or a greater value. The fact that many plants sometimes escape without a single visible lesion, despite apparently optimum conditions for infection, while others are severely attacked, presents a baffling and difficult problem. Thus,

TABLE I

EFFECT OF SOIL MOISTURE AND SOIL TEMPERATURE ON DEGREE AND TYPE OF SPROUT CANKER OF POTATO CAUSED BY *Rhizoctonia Solani* IN SOIL ARTIFICIALLY INFESTED

Exp.	Moisture, %†	°C.	Injury		% Plants escaped	Isolate
			%	Type‡		
1	21	16	50	T	6	76
	27	16	60	T	0	
	38	16	60	T	0	
	21	23	60	T	0	
	27	23	57	T	0	
	38	23	45	T	25	
2	21	16	50	T	20	76
	29	16	63	T	0	
	39	16	51	T	0	
	21	23	39	T-S	20	
	29	23	52	T	0	
	39	23	50	T	0	
3	20	16	18	T	66	76
	39	16	3	T	94	
4	20	16	66	T	0	76
	38	16	66	T	6	
	19	23	18	S	60	
	36	23	6	S	31	
5	31	18	70	T	16	76
	39	18	38	T-S	20	76
	31	18	50	T	10	108
	39	18	40	S	7	108
6	19	18	16	S	75	76
	19	18	5	S	90	106
	27	18	2	S	85	76
	27	18	0		100	106
	36	18	20	S	40	76
	36	18	0		100	106

Exp.	Moisture, %†	°C.	Injury		% Plants escaped	Isolate
			%	Type‡		
7	18	17	47	T	17	76
	39	17	20	T	67	
8	23	17	80	T	0	76
	39	17	53	T	0	
9	23	20	80	T	0	76
	42	20	25	S	10	
10	29	20	80	T	0	76
	28	25	10	S	40	
11	31	20	90	T	0	76
	29	25	41	T-S	0	
12						
ck.	32	16	50	T	13	76
A	32	16	10	S	40	
ck.	30	23	50	T	19	
A	30	23	55	T	0	
13						
ck.	33	16	31	T-S	50	76
A	33	16	12	S	50	
B	33	16	2	S	44	
C	33	16	5	S	66	
ck.	31	23	32	S-T	0	
A	31	23	56	T	10	
B	31	23	55	T	0	
C	31	23	36	T	41	

† *At conclusion of test, on basis of moisture-holding capacity of soil. During 21 days of the test about 1.5%, 2%, and up to 3% was lost from the dry, medium and wet series, respectively.*

‡ *"T" indicates prevalence of destruction of first sprout tip, and "S" prevalence of lesions on side of sprout.*

although a disease rating below, say 40%, is usually associated with the less virulent type of attack, it may be because only a few plants carried evidence of the "T" type of attack, and the others wholly escaped.

A point to be kept in mind is that all tests listed in Tables I and II were made in evenly and thoroughly infested natural soil for the duration of 21 days after the sets were planted. This method appeared adequate for the purpose of the study.

The year and month in which each of the 13 experiments listed in Table I were made are as follows: in 1937, October, Nos. 1 and 2; March, Nos. 3, 4 and 5; in 1936, March, No. 6; April, No. 7; May, Nos. 8, 9 and 10; July, No. 11; October, No. 12, and November, No. 13.

In Experiment 1, Table I, the attack appeared equally severe in the dry, medium and wet soils. The severe "T", or primary sprout type of attack, prevailed at both 16° and 23° C., and, with the exception of two units of the experiment, all the plants were affected. It is of interest to note that 25% of the plants in the soil at 38% moisture-holding capacity escaped attack, although the others were severely injured. Also, despite more rapid growth of the sprouts in the wet soil at 23°, and what might appear a greater tendency to recover, they were, on the whole, equally severely attacked. A careful examination of all the different series from Experiment 1, shown in Plate I, will confirm this analysis. In Experiment 2, a similar situation prevailed. The lower figures for the dry sets at 16° and 23° are accounted for by 20% of the plants escaping attack in each case.

The results of Experiment 3 appear to indicate more disease (18%) in the dry soil than (3%) in the wet one at 16°. In both dry and wet soils, the primary sprouts of affected plants were destroyed, but a large percentage of the plants in each case simply escaped. The results of Experiment 4 are practically identical at 16° in dry and wet soils, with the "T" type of injury prevailing, but at 23° the attack, which was of the "S" type and not severe, was definitely greater in the dry soil, despite the fact that twice as many plants escaped. In this case the injury was much more severe at 16° than at 23°; this is confirmed by the corresponding percentage of plants that escaped.

In Experiment 5, in which the soil temperature was 18°, and two isolates of contrasting pathogenicity employed, the attack of No. 76, a virulent isolate, was definitely greater at medium soil moisture (31% moisture-holding capacity) than in the wet soil (39%), and for isolate No. 108, also probably somewhat greater in the former soil. The results of Experiment 6, the soil temperature of which was 18°, indicate no definite difference in severity between the dry and wet soil. Practically no disease developed in the soil of medium moisture; for this no explanation is available. However, with *R. Solani*, variation of this kind may be expected, despite apparently rigid control.

In Experiment 7, in which the temperature was 17°, more disease occurred in the dry soil than in the wet one. However, it will be observed that the "T" type of injury prevailed in both cases, but about four times more plants

escaped in the wet soil. Experiment 8, at 17°, concluded the following month, indicated great severity of disease in a soil the moisture content of which was between dry and medium, *viz.*, 23% moisture-holding capacity, and 27% less injury in one of 39% moisture-holding capacity. No plants escaped, and the "T" type of injury prevailed. Another experiment (No. 9) at 20°, instead of 17°, again indicated practically maximum severity with the "T" type of injury at 23% soil moisture content, and 55% less disease with the "S" type of lesions at 42% soil moisture.

The results of Experiment 10 indicate that in a soil of medium moisture (28–29%) approximately maximum injury of "T" type occurred at 20°, while at 25° it was at least eight times less severe, with 40% of the plants escaping and an absence of the "T" type of injury. Experiment 11, made a month later, provides results which, for the medium moist soil, practically duplicate those of Experiment 10, although there was nearly one-half as much (40%) injury at 25° as there was at 20°, with an equal amount of plants affected with each type of injury.

Experiments 12 and 13 are similar and concern another phase of the general study in progress. The results are given here in connection with the temperature and moisture studies for the purpose of indicating the possible effect of other factors, such as nutrients, in modifying the severity of the disease at a given soil temperature or soil moisture. To Series A, B and C, of both experiments indicated, were added different amounts of sodium nitrate. The checks received none. The soil moisture was, in general, slightly above optimum, or between 30 and 33% moisture-holding capacity. In Experiment 12, the disease at 23° in the A series was five times more severe than in the corresponding series at 16°, and in Experiment 13, carried out one month later, it was again about five times greater at 23°. In Series B and C of Experiment 13, the increase was roughly 27 and 7 times, respectively, over that at 16°. In the controls of each experiment the disease was about equally severe at 16° and 23°. No particular significance should be attached to the greater severity of the disease in the checks of Experiment 12 than in those of Experiment 13, but it is important to note that the severity was practically the same in the checks at 16° and 23° in each of the two cases.

Table II provides additional data on the effect of soil temperatures of 17° and 23°. The soil moisture was approximately 29% moisture-holding capacity, or about optimum. With the exception of No. 76, the 25 random isolates indicated were tested at one time. Temperature was the only known variable. The apparent difference in pathogenic capability of this and other isolates (13 in all) is discussed in the previous paper (14).

Of the 26 isolates listed, nine produced more disease at 17°, and six of them (Nos. 1, 7, 10, 13, 14, and 15) caused more injury at 23°. The difference may not have been significant in all cases mentioned. Nine of the remaining 11 isolates were about equally pathogenic at 17° and 23°, and two did not produce disease. Possibly the "T" type of injury was more common at 17° than at 23°, but in certain cases both "T" and "S" types were about equally prevalent at both temperatures.

TABLE II

THE RELATIVE DISEASE INJURY TO POTATO SPROUTS, CAUSED BY VARIOUS RANDOM ISOLATES OF *Rhizoctonia Solani* FROM POTATO TUBERS, AT 17° AND 23° C. AND SOIL MOISTURE CONTENT APPROXIMATELY 27–29% MOISTURE-HOLDING CAPACITY.

Isolate	17° C.			23° C.			Isolate	17° C.			23° C.		
	Injury		% Plants escaped	Injury		% Plants escaped		Injury		% Plants escaped	Injury		% Plants escaped
	%	Type†		%	Type†			%	Type†		%	Type†	
1	41	T-S	25	66	T	0	14	8	T	81	28	S	31
2	43	S-T	12	32	S-T	31	15	26	T	50	32	S-T	31
3	33	T-S	25	7	S	81	16	45	T	25	36	T-S	19
4	11	S	62	12	S	62	17	1	S	81	0		100
5	0		100	0		100	18	36	T	31	10	S-T	81
6	28	S	6	18	S	31	19	32	T-S	31	24	T-S	50
7	7	S	75	19	S	25	20	2	S	81	0		100
8	27	T-S	44	31	S-T	38	21	44	T	19	26	S-T	50
9	0		100	0		100	22	10	T	62	10	S	56
10	0		100	27	S	31	23	5	S-T	81	4	T	88
11	18	S	31	7	S	81	24	30	T-S	44	10	S	75
12	0		100	2	S	88	25	6	S	81	6	S	94
13	16	S	62	44	T	6	76‡	60	T	0	59	T	0

† *"T" and "S" indicate relative prevalence of destruction of primary sprout, and lesions on side of stems, respectively.*

‡ *See Experiment 1, Table I.*

Growth Rate of Pathogen in Relation to Temperature and Moisture

It has frequently been assumed that an abundant and rapid growth of the pathogen has an important bearing on the development of the disease. In testing 133 isolates for pathogenicity to the potato it was observed (14) that some isolates characteristically made more abundant and quicker growth than others did. However, very frequently the severity of disease seemed to bear a negative relation rather than a positive one to abundant growth. But, according to the data just presented, the disease may develop to maximum severity at 16° and 17° C., and surprisingly well in the very dry soil, where less growth is expected.

On a Nutrient Medium

It was decided to compare, on a nutrient medium, the rate of growth of 12 representative isolates, six of which proved pathogenic to potato, and six of which did not. These isolates were incubated at 10°, 15°, 20° and 25° C. in Petri plates on potato-dextrose agar, and the daily rate of growth was measured during ten days. The growth of these isolates, based on the measurements from six plates during four days, is compared in Fig. 1. With certain exceptions, the data indicated that for the fungus to grow the same distance covered in four days at 25°, it would require slightly over five days at 20°, about six days at 16°, and ten days at 10°. However, pathogenic Isolates Nos. 76 and 20, and non-pathogenic Isolate No. 48, grew almost equally well at 20° and 25°. Also, certain isolates apparently were relatively more or less vigorous than others at a given temperature. This may explain why a more uniform agree-

ment with regard to optimum temperature for growth has not been reached by certain other investigators. There seems no doubt that the optimal point for the great majority of the isolates pathogenic to the potato is somewhat above 20°, and probably very near 25°. Some dissimilarity among isolates in this respect is to be expected.

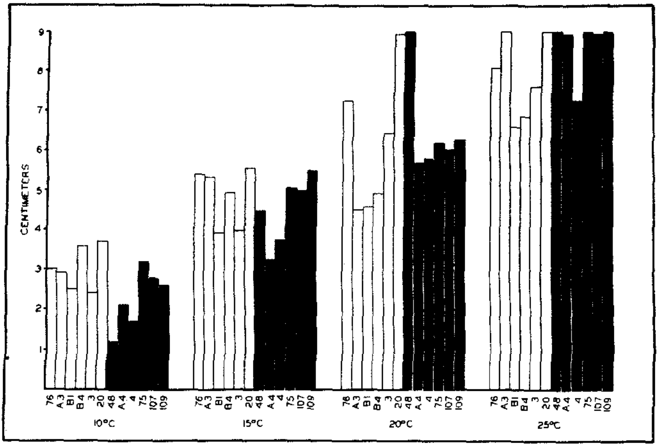

FIG. 1. *The relative growth rate during four days of 12 isolates of Rhizoctonia Solani on potato-dextrose agar at 10°, 15°, 20° and 25°C. The unshaded columns represent virulent isolates, and the shaded ones non-pathogenic isolates.*

Growth Rate of Pathogen in Soil

This was determined by measuring the daily growth of *R. Solani* in steam sterilized, black loam soil, described as Type 1 in the previous paper (14). The soil, adjusted to approximately 20% (dry), 30% (medium), or 39% (wet), on the basis of moisture-holding capacity, was placed in large test tubes, which were loosely plugged and steam sterilized, after which they were inoculated and incubated at 16° and 23°. There were ten soil tubes in each test. The average rate of growth for each isolate during ten days is charted in Fig. 2.

The rate of growth in the wet soils at 23°, and also at 16°, slightly exceeded that in the corresponding soils of medium moisture content. Slowest growth occurred in the dry soil at both temperatures. Evidently a moisture content of 20% moisture-holding capacity, which is still sufficient for fair growth of the potato plant, was more important in determining the growth rate of the pathogen than the temperature factor itself between 16° and 23°.

Discussion

We may now examine the effect on the disease of soil moisture and soil temperature in the following connections, *viz.*, on the growth rate of *R. Solani;* on the severity and type of attack on potato shoots; and, finally, on the ability of the host to recover from attack.

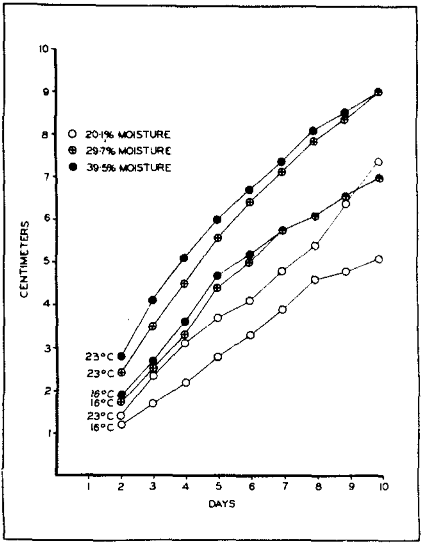

FIG. 2. *The relative growth rate of Rhizoctonia Solani during ten days in sterilized soil at 16° and 23° C., and soil moisture contents of 20.1%, 29.7%, and 39.5%.*

The growth rate of *R. Solani* in a soil is modified by temperature and very greatly by the moisture content. The optimum temperature for nine of the 12 isolates tested seemed near 25°, but three of them appeared to grow as well at 20° as at 25°. Therefore, it is obvious that the optimum temperature for *R. Solani* cannot be determined from a single isolate. Again, it is characteristic of some isolates to grow slowly, while others grow quickly and abundantly. In dry soils the latter type would seem more effective pathogens than the former, if the limiting factor is only a matter of distance between the host and pathogen. On the contrary, these inherent differences in growth rate among isolates, although effective in controlled experiments, may easily be of little or no practical importance under field conditions. It has been pointed out previously in these studies that the usual differences in growth

rate which occur at temperatures of 23° and 16° in a moist soil are practically nullified if the soil is dry. This is significant because it indicates that a dry osil of 19% moisture-holding capacity is more important in reducing the growth rate, and therefore chances of infection, than the low temperature of 16°.

The evidence regarding the effect of soil moisture on the severity of disease and type of injury (Tables I and II) indicates that while there may be a slight tendency for greater severity in a soil deficient in moisture than in one of moderate or high moisture content, the difference was not definite and constant enough to warrant a final conclusion. However, it is certain that, as a rule, the disease can attain maximum severity on young potato sprouts in an unsterilized soil artificially infested with the pathogen at soil moisture contents ranging between a fairly dry state and a fairly wet condition, *viz.*, 19% and 40% moisture-holding capacity, respectively. The effect of rather limited soil moisture *versus* an adequate supply, on recovery of the host from the first attack by means of secondary sprouts, is discussed in another paragraph.

Expected differences with regard to the differential effect on disease severity and type of attack, of temperatures between 16° and 23°, inclusive, were not obtained in these studies. At 25° the severity abruptly decreased, but at 23° the pathogen appeared as frequently virulent as at 16°, 18° or 20°, and the type of injury was not essentially different. Apparently, under the conditions of the test, striking and consistent differences are not to be expected; otherwise at least a tendency in certain directions would have been indicated. Perhaps under other conditions the differential effect of temperatures between 16° and 23° could be demonstrated, but the present studies suggest the advisability of at least several tests for a decision.

Richards (12) believed that the "T" type of injury was favored by soil temperatures of 18° and somewhat lower, and the "S" type by temperatures near 23°. The data of this paper indicate that either type of injury could occur to the practical exclusion of the other at both 23° and 16°. The prevalence of the "T" type of injury at any temperature merely indicates that the environmental factors favored a high degree of virulence of the pathogen on the one hand, or the susceptibility of the host on the other. Likewise the "S" type is an indication of weak parasitism.

Undoubtedly there are other factors, besides temperature and moisture, which play very important roles in the parasitism of *R. Solani* to the potato. Whether these affect the pathogen or the host, or both, is not clear. Certain incomplete evidence at hand suggests that the young host sprout, as affected by storage conditions, may be more susceptible at one time than another. Again, the pathogen, in the presence of certain nutrients, as, for example, sodium nitrate, produced more disease at 23° than at 16° (Experiments Nos. 12 and 13, Table I). Also, greater parasitism was exhibited in an infertile podsol soil than in a fertile organic loam (14).

Finally, there is the question of growth rate of the host in relation to the type of attack and the degree of injury, suggested by Richards (12). Given equal soil moisture, the emergence of the primary sprout requires less time, and its subsequent growth is considerably better at 23° than at 16°. The

greatest contrast in growth rate is found between a wet soil at 23° and a dry one at 16°. Thus, if the disease tends to be more severe at the lower temperature range, as indicated by Richards (12) and Dana (2), and if increased disease severity is really associated with slow growth, then a dry soil at low temperature should favor the attack most. But these studies have failed to demonstrate a constant and definite tendency in this respect.

The effort at recovery by means of secondary sprouts from severely lesioned primary sprouts was a characteristic feature. Naturally they were larger at 23° and also grew more quickly than at 16°. In soils of wet and medium moisture their number was not noticeably different at these temperatures but in dry soil the number tended to be limited to one, particularly at 16° (Plate I). Thus, if, toward the end of the first 21 days after planting, these sprouts are an important factor in recovery, less progress is made in a dry soil at a low temperature. But this phenomenon, although economically valuable, should not be confused with a consideration of parasitism itself. However, it is both important and interesting that a high percentage of these secondary sprouts appear to possess a remarkable degree of resistance, notwithstanding the primary sprouts were very susceptible and severely attacked under apparently identical conditions. Their escape seems to be equally common at 16° and 23° (Plate I).

A general conclusion from this study is that, heretofore, probably too much emphasis has been given to the effect of temperature between 16° and 23°, and not enough attention to other factors which affect the ability of the pathogen to attack, and the host to resist.

Acknowledgment

The assistance of Mr. G. M. Tosh, Student Assistant at this laboratory, with the photographs, charts and experimental work is acknowledged with appreciation.

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AGRICULTURAL METEOROLOGY: CORRELATION OF MONTHLY PRECIPITATION IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN WITH LATITUDE, LONGITUDE AND ALTITUDE[1]

By J. W. Hopkins[2]

Abstract

The linear partial regression coefficients of the 19-year average (1917–1935) *monthly precipitation recorded at 42 points in central and southern Alberta* on latitude, longitude and altitude were determined for each month of the year. The correlation of precipitation with these co-ordinates, although statistically significant, was only moderate. Some improvement was effected by inclusion of the quadratic term in longitude, but even so, more than 50% of the inter-station variance of the 19-year precipitation averages for most months remained in the form of residual deviations. Observations for individual years were even less *amenable to graduation. Consequently, a given number of meteorological* stations would provide a much less complete specification of precipitation than of air temperature (the subject of a parallel previous study) within the area considered.

In a previous communication (3), the writer described the correlation of monthly mean air temperature with the latitude, longitude and altitude of meteorological stations in central and southern Alberta and Saskatchewan, and indicated the significance of the correlations found in connection with a criterion of the adequacy of the number of meteorological stations within an area, proposed by Irwin (4). The present paper gives the results of a parallel study of precipitation statistics for stations in the same districts. As before, two series of data are considered, *(a)* long-term or climatological averages, and *(b)* records for an individual year.

Data

For the climatological series, it was planned to use the precipitation records for the set of 43 stations adopted in the preceding study of air temperature, as this would have reduced to a minimum the further arithmetical work required in the calculation of the regression coefficients. When, however, the available monthly totals of precipitation for the 19-year period 1917–1935 were extracted from the Monthly Record published by the Meteorological Service of Canada (5), it was found that there were numerous discontinuities in the data for Shaunavon, Saskatchewan, and this station had in consequence to be discarded. Table I shows the 19-year average of precipitation, expressed as inches of rain (10 in. snow = 1 in. rain), for each month of the year at the remaining 42 stations. (Some substitutions of observations at neighboring points were necessary in the earlier years). The mean values given at the foot of this table show a pronounced seasonal trend, the average precipitation

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[2] *Statistician, National Research Laboratories, Ottawa.*

TABLE I

Average monthly precipitation (as inches of rain), 1917–1935

Station	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Alberta												
Alix	0.82	0.64	1.12	1.23	1.79	2.56	2.26	1.93	1.41	0.74	0.82	0.81
Bassano	.49	.48	.64	1.36	1.91	1.85	1.85	1.17	1.21	.55	.58	.62
Calgary	.47	.59	1.03	1.41	1.54	3.20	2.31	1.92	1.84	.81	.71	.76
Calmar	.70	.61	.92	1.20	2.36	3.16	2.68	2.71	1.55	.84	.80	.84
Edmonton	.99	.66	.97	1.06	1.90	2.87	2.99	2.29	1.17	.80	.84	.97
Gleichen	.62	.66	.77	1.33	1.46	2.19	2.14	1.50	1.28	.65	.69	.76
Harmattan	.59	.52	.96	1.36	2.07	2.97	2.58	2.78	1.91	.74	.65	.68
High River	.92	.97	1.82	2.01	1.87	3.55	1.91	2.12	1.75	1.39	1.09	1.05
Hillsdown	.72	.57	.96	1.46	1.53	2.13	1.82	1.57	1.50	.72	.71	.76
Lacombe	.63	.61	.83	1.45	2.03	2.77	2.51	2.34	1.70	.78	.77	.83
Lethbridge	.50	.66	1.07	1.58	1.76	2.38	1.51	1.37	1.76	1.01	.85	.83
Lundbreck	.76	.92	1.45	1.40	1.59	3.03	1.70	1.72	1.25	1.00	1.01	1.09
Macleod	.64	.67	1.15	1.42	1.66	2.71	1.62	1.45	1.75	1.07	.86	.78
Medicine Hat	.67	.40	.69	1.12	1.28	2.09	1.51	1.34	1.28	.65	.56	.85
Olds	.59	.67	.79	1.43	2.01	2.99	2.27	2.52	1.66	.69	.58	.59
Pekisko	1.05	.87	1.59	2.26	2.48	3.70	1.97	2.43	2.50	2.52	1.32	1.22
Perbeck	.51	.36	.67	.98	1.81	2.80	2.29	1.74	1.58	.54	.45	.56
Ranfurly	.90	.83	1.17	.98	1.85	2.61	2.50	2.34	1.26	.89	.75	.86
Strathmore	.39	.60	.64	1.29	1.88	2.85	1.77	1.94	1.60	.79	.72	.53
Saskatchewan												
Anglia	.56	.35	.65	.83	1.20	2.14	1.56	1.43	1.03	.71	.47	.43
Battleford	.61	.37	.48	.98	1.29	2.44	1.78	1.71	1.41	.73	.44	.53
Chaplin	.55	.43	.68	.89	1.69	2.77	1.80	1.79	1.15	.80	.52	.45
Ft. Qu'Appelle	.73	.52	.79	.79	1.49	2.75	2.09	1.72	1.34	.98	.80	.53
Indian Head	.83	.62	1.24	.99	1.76	3.08	2.35	1.78	1.55	1.16	1.03	.72
Kamsack	.98	.60	.78	.77	1.41	3.14	2.50	2.12	1.42	.91	.64	.67
Klintonel	.87	.79	1.04	1.47	1.89	3.08	2.04	1.48	1.37	.80	.72	.88
Melfort	.65	.40	.69	.75	1.41	2.66	2.13	2.21	2.19	1.03	.77	.54
Moose Jaw	.65	.58	.79	.91	1.68	3.02	1.67	1.90	1.50	.93	.77	.69
Muenster	.62	.33	.72	.82	1.56	3.48	2.41	1.76	1.65	.89	.47	.58
Nashlyn	.39	.39	.45	.94	1.29	2.13	1.57	.95	.96	.39	.52	.50
Pilger	.73	.52	1.01	.86	1.31	3.12	2.22	1.68	1.63	.84	.58	.63
Prince Albert	.61	.42	.90	1.08	1.40	3.06	1.95	2.18	1.73	.90	.66	.67
Qu'Appelle	.87	.62	1.18	1.32	1.82	3.37	2.28	1.89	1.64	1.29	1.08	.68
Regina	.66	.42	.78	.79	1.55	3.08	2.03	1.65	1.28	.98	.80	.48
Rosthern	.62	.45	.91	.98	1.33	2.61	2.31	1.78	1.51	.94	.58	.61
St. Walburg	.76	.53	.71	.98	1.33	2.57	2.60	1.97	1.30	.64	.59	.58
Saskatoon	.66	.48	.84	1.03	1.22	2.76	2.33	1.70	1.52	.97	.65	.53
Scott	.65	.55	.74	1.00	1.16	2.26	1.96	1.52	1.24	.60	.56	.67
Swift Current	.73	.50	.67	.93	1.65	2.94	2.10	1.95	1.34	.83	.54	.64
Waseca	.82	.65	.71	1.11	1.22	2.37	1.81	1.76	1.42	.61	.77	.61
Whitewood	.92	.49	.84	1.20	1.75	3.23	2.24	1.78	1.66	1.23	.97	.68
Yellow Grass	.65	.57	.77	1.06	1.74	3.05	2.06	1.74	1.52	.90	.82	.59
Mean	0.69	0.57	0.90	1.16	1.64	2.80	2.09	1.85	1.51	0.89	0.73	0.70
Standard deviation	.16	.15	.28	.32	.31	.43	.35	.39	.29	.33	.19	.17
Standard deviation, %	23	26	31	28	19	15	17	21	19	37	26	24

for the 42 stations being greatest in June and least in February. Absolute variation between stations in the 19-year average, as indicated by the standard deviation, is likewise greatest in summer and least in winter, but the relative variability tends to be greatest in winter.

Analysis of Climatological Series

The latitude, longitude and altitude of the 42 meteorological stations may be found in Table I of the preceding paper (3), and accordingly are not repeated here. By using the values given there, the Normal Equations to determine the linear regression coefficients b_1, b_2, and b_3 of January precipitation on latitude, longitude and altitude were found to be

$$\begin{aligned} 239{,}747\, b_1 + 43{,}485\, b_2 - 982{,}441\, b_3 &= 61.92 \\ 43{,}485\, b_1 + 2{,}452{,}267\, b_2 + 5{,}885{,}396\, b_3 &= -255.06 \\ -982{,}441\, b_1 + 5{,}885{,}396\, b_2 + 24{,}014{,}360\, b_3 &= -82.90 \end{aligned}$$

the left-hand side differing slightly from that of the corresponding equations in the earlier paper, owing to the omission of Shaunavon. The equations for the other 11 months differed from the foregoing only in the substitution on the right-hand side of the successive trios of products:

−85.46	−149.70	−286.84	−126.85	−138.11	634.38
697.47	833.09	2116.83	1409.49	−821.78	−122.22
2881.63	4276.18	8222.90	5389.18	2097.71	−1886.01

594.02	34.00	−244.46	−178.67	−71.25
768.67	346.51	−213.23	189.58	925.35
1236.36	2839.55	2967.72	2234.59	3266.46

Solution of these 12 sets of equations was again effected by means of Fisher's inverse matrix method (1, Sec. 29), the multipliers being, in millionths,

	$c_{.1}$	$c_{.2}$	$c_{.3}$
$c_{1.}$	8.365264	−2.354635	0.919298
$c_{2.}$		1.652989	−0.501441
$c_{3.}$			0.202143

By the use of these multipliers, the regression coefficients and their respective standard errors listed in Table II were arrived at. Table III shows the

TABLE II

PARTIAL REGRESSION COEFFICIENTS OF 19-YEAR AVERAGE (1917–1935) MONTHLY PRECIPITATION (EXPRESSED AS INCHES OF RAIN) ON LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS

Month	Partial regression coefficient of precipitation on		
	Latitude (1/100's in. per 10′ N.)	Longitude (1/100's in. per 10′ W.)	Altitude (1/100's in. per 100 ft.)
January	+1.04 ± .43	−0.53 ± .19	+1.68 ± .67
February	+ .29 ± .36	− .09 ± .16	+1.54 ± .56
March	+ .72 ± .71	− .41 ± .32	+3.09 ± 1.10
April	+ .18 ± .55	+ .05 ± .24	+3.37 ± .86
May	+ .57 ± .76	− .07 ± .34	+2.66 ± 1.19
June	+2.71 ± 1.02	−2.08 ± .45	+7.09 ± 1.58
July	+3.86 ± .81	− .75 ± .36	+2.63 ± 1.26
August	+4.30 ± .91	− .75 ± .40	+4.10 ± 1.41
September	+2.08 ± .74	− .93 ± .33	+4.31 ± 1.15
October	+1.19 ± .81	−1.26 ± .36	+4.82 ± 1.27
November	+ .11 ± .51	− .39 ± .23	+1.92 ± .79
December	+ .23 ± .42	+ .05 ± .19	+1.31 ± .65

coefficient of multiple correlation R between precipitation and latitude, longitude and altitude for each of the 12 months, and also the residual standard deviation of the actual 19-year station averages from the graduated values of the regression equation for each month.

TABLE III

MULTIPLE CORRELATION COEFFICIENT (R) BETWEEN 19-YEAR AVERAGE (1917–1935) PRECIPITATION AND LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS, AND RESIDUAL STANDARD DEVIATION (s), BY MONTHS

Month	R	s, in.
January	0.43	0.15
February	.61	.13
March	.52	.25
April	.82	.19
May	.57	.26
June	.61	.35
July	.64	.28
August	.63	.31
September	.53	.26
October	.56	.28
November	.47	.18
December	.61	.14

It may be noted at once that inter-station differences in the 19-year averages of monthly precipitation are much less closely representable by linear functions of the co-ordinates of position and altitude than were the corresponding differences in air temperature (3). However, the variation between stations in respect to precipitation is not entirely unsystematic or non-linear. Thus the occurrence of 12 coefficients of like sign, as in the second column of Table II, from 12 uncorrelated series is an event having a random probability of only 1 in 2048, from which it may be inferred with reasonable certainty that there is a definite tendency for the amount of precipitation to increase from south to north. Similarly, the 19-year average decreases somewhat from east to west, after allowance is made for differences in latitude and altitude. In both cases, the maximum trends, and indeed almost the only ones of any significance, occur during the period June-October. Of the three co-ordinates, however, that of altitude is most consistently associated with differences in precipitation, the regression coefficients being again all positive (*i.e.*, precipitation increasing with altitude), and 11 of the 12 being individually statistically significant. Nevertheless, even when all three factors are taken into account, the multiple correlation coefficients shown in Table III are generally very moderate, and in only a few months is the standard deviation appreciably reduced by the linear graduation effected. This is in marked contrast to the results previously obtained in the analysis of the corresponding temperature data (3).

When the residual deviations of the actual from the graduated values of precipitation were being computed, it was noted that certain stations tended to have a majority of negative, and others to have a majority of positive deviations. The algebraic sums of the deviations for each station for the separate six-month periods April-September and October-March were entered accordingly upon maps of central and southern Alberta and Saskatchewan. In this way, it was found that the positive deviations tended to be concentrated in the eastern and western quarters of the area, whereas the

central half had a predominance of negative deviations. This, of course, suggested that even after allowing for differences in latitude and altitude, precipitation did not, on the average, increase steadily from east to west, but was at a minimum somewhere in the central region. It seemed worthwhile, therefore, to ascertain whether inclusion of the quadratic term in longitude in the regression equation would lead to a significant improvement in graduation.

This necessitated the calculation of a number of additional sums of squares and products, after which the Normal Equations to determine the regression coefficients b_1, b_2, b_3, and b_4 of January precipitation on latitude, longitude, altitude and the square of longitude ($\times$ 10^{-2}) were found to be:

$$
\begin{aligned}
239{,}747\, b_1 + 43{,}485\, b_2 - 982{,}441\, b_3 + 193{,}308\, b_4 &= 61.92 \\
43{,}485\, b_1 + 2{,}452{,}267\, b_2 + 5{,}885{,}396\, b_3 + 23{,}252{,}993\, b_4 &= -255.06 \\
-982{,}441\, b_1 + 5{,}885{,}396\, b_2 + 24{,}014{,}360\, b_3 + 58{,}644{,}655\, b_4 &= -82.90 \\
193{,}308\, b_1 + 23{,}252{,}993\, b_2 + 58{,}644{,}655\, b_3 + 228{,}141{,}509\, b_4 &= -1456.67
\end{aligned}
$$

the equations for the other 11 months being again derived from these by the substitution on the right-hand side of the successive quartets of sums of products:

−85.46	−149.70	−286.84	−126.85	−138.11	634.38
697.47	833.09	2116.83	1409.49	−821.78	−122.22
2881.63	4276.18	8222.90	5389.18	2097.71	−1886.01
7480.39	9898.89	21773.53	16028.56	−3707.52	1011.22

594.02	34.00	−244.46	−178.67	−71.25
768.67	346.51	−213.23	189.58	925.35
1236.36	2839.55	2967.72	2234.59	3266.46
10274.82	5711.97	558.47	3683.56	9766.05

TABLE IV

PARTIAL REGRESSION COEFFICIENTS OF MEAN MONTHLY PRECIPITATION (1917–1935) AT METEOROLOGICAL STATIONS IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN ON LATITUDE (b_1), LONGITUDE (b_2), ALTITUDE (b_3) AND THE SQUARE OF LONGITUDE (b_4)

Month	b_1, 1/100's in. per 10′ N.	b_2, 1/100's in. per 10′ W.	b_3, 1/100's in. per 100 ft.	b_4, 1/100's in. per (10′ W.)^2
January	0.96	−1.43	1.28	.0105
February	.24	− .72	1.27	.0073
March	.57	−2.04	2.39	.0189
April	.09	− .94	2.94	.0115
May	.34	−2.65	1.54	.0301
June	2.40	−5.46	5.62	.0394
July	3.60	−3.63	1.39	.0335
August	3.98	−4.25	2.59	.0408
September	1.89	−3.03	3.40	.0245
October	1.02	−3.13	4.01	.0217
November	−.04	−2.12	1.17	.0201
December	.15	− .79	.94	.0099

TABLE V

ANALYSIS OF VARIANCE, REGRESSION OF MEAN MONTHLY PRECIPITATION (1917–1935) AT METEOROLOGICAL STATIONS IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN ON LATITUDE, *LONGITUDE AND ALTITUDE*

Variance due to	Degrees of freedom	January		February		March		April		May		June	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square
Longitude (quadratic)	1	0.0752	0.0752	0.0359	0.0359	0.2417	0.2417*	0.0899	0.0899	0.6116	0.6116**	1.0502	1.0502**
Latitude, longitude and altitude (linear)	3	0.1847	0.0616*	0.3560	0.1187**	0.8687	0.2896**	2.8295	0.9432**	1.2566	0.4189**	2.8270	0.9423**
Residual	37	0.7629	0.0206	0.5589	0.0151	2.0418	0.0552	1.2887	0.0348	2.0365	0.0551	3.6445	0.0985
Total	41	1.0228	–	0.9508	–	3.1522	–	4.2081	–	3.9047	–	7.5217	–

Variance due to	Degrees of freedom	July		August		*September*		*October*		*November*		*December*	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square
Longitude (quadratic)	1	0.7605	0.7605**	1.1278	1.1278**	0.4059	0.4059*	0.3200	0.3200*	0.2752	0.2752**	0.0664	0.0664
Latitude, longitude and altitude (linear)	3	2.0444	0.6815**	2.4844	0.8281**	0.9733	0.3244**	1.4107	0.4702**	0.3364	0.1121**	0.4659	0.1553**
Residual	37	2.2257	0.0602	2.6330	0.0712	2.0942	0.0566	2.6916	0.0727	0.9130	0.0247	0.7216	0.0195
Total	41	5.0306	–	6.2452	–	3.4734	–	4.4223	–	1.5246	–	1.2539	–

* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

Proceeding as before, the matrix of multipliers for the solution of these 12 sets of equations was found to be, in millionths:

	$c_{.1}$	$c_{.2}$	$c_{.3}$	$c_{.4}$
$c_{1.}$	8.45445	−1.37015	0.96220	−0.11481
$c_{2.}$		12.52292	−0.02949	−1.26764
$c_{3.}$			0.22263	−0.05504
$c_{4.}$				0.14783

These gave the partial regression coefficients of mean monthly precipitation on latitude, longitude, altitude and the square of longitude ($\times 10^{-2}$) shown in Table IV.

The improvement in graduation resulting from the inclusion of the quadratic term may be determined from the additional variance accounted for. This is indicated in Table V. For all 12 months the variance accounted for by the additional term exceeds the mean square residual, so that the results as a whole are certainly consistent with the supposition of a non-linear element in the longitudinal variation of precipitation. Considering the data for each month individually, however, it is seen that during the winter months, when the inter-station variation in precipitation is least, the increase in precision is not significant.

The regression coefficients for altitude in Table IV are again all positive, indicating that, on the average, precipitation increases with elevation. There is likewise a definite tendency for precipitation to increase from south to north, but this is much more pronounced in the summer than in the winter months. The amounts actually received increase, on the whole, from east to west, but it was indicated in Table II that this was more than accounted for by differences in altitude, so that after allowing for this factor the linear regression of precipitation on west longitude was either insignificant or negative. The linear coefficients b_2 of Table IV are again uniformly negative, but the quadratic coefficients b_4 are all positive, indicating that the longitudinal variation passes through a minimum somewhere in the central part of the region. The actual longitude λ corresponding to the minimum of the longitudinal graduation was readily computed by equating the partial derivative of the regression equation for each month with respect to longitude, namely $b_2 + 2/10\lambda b_4$, to zero. The

TABLE VI

MULTIPLE CORRELATION COEFFICIENT (R) BETWEEN MEAN PRECIPITATION, 1917–1935, AND LATITUDE, LONGITUDE AND ALTITUDE, AND RESIDUAL DEVIATION (s), BY MONTHS

Month	R	s, inches	s, per cent
January	0.50	0.14	21
February	.64	.12	22
March	.59	.24	26
April	.83	.19	16
May	.69	.24	14
June	.72	.31	11
July	.75	.25	12
August	.76	.27	14
September	.63	.24	16
October	.63	.27	30
November	.63	.16	22
December	.65	.14	20

twelve values thus obtained fluctuated irregularly about a mean of 110° 9′ (*i.e.*, the meridian passing about 30 miles east of Medicine Hat), with no clear indication of systematic seasonal variation.

Table VI shows for each month the multiple correlation coefficient R between mean monthly precipitation and latitude, longitude (quadratic) and altitude, and the residual deviation s of the actual from the graduated values both in inches of rain and as a percentage of the 19-year 42-station mean. Even after the inclusion of the quadratic term, the agreement between the actual and graduated values leaves a good deal to be desired, more than 50% of the inter-station variance of the 19-year averages for most months remaining in the form of residual deviations. A given number of meteorological stations would thus provide a much less complete specification of precipitation than of air temperature within the area considered.

Results for Individual Years

As in the case of air temperature (3), when the precipitation during any one month of a single year, rather than the monthly average for the 19-year period, is considered, further variation may arise from two sources. (i) Over the area as a whole, the precipitation of the month in question may be above or below the climatological average. (ii) Local variations, which tend to offset each other as the number of years' records increases, will be more pronounced. Annual differences of Type (i), in so far as they affect all stations equally, need not increase the residual standard deviation, since they require only an adjustment in the constant term of the regression equation. Irregular local variations, on the other hand, will, of course, result in greater discrepancies between the observed and graduated values.

The actual extent of such effects in the data for the months of January and July was investigated by the analysis of variance procedure (1). In each case, the total variance of the 19 × 42 monthly totals of precipitation was subdivided into portions due to (i) differences between the 19-year averages of the 42 individual stations; (ii) differences between the 42-station averages of the 19 individual years; and (iii) residual irregular variation. The results of these computations are shown in Table VII.

TABLE VII

Analysis of variance of January and July precipitation (total inches of rain) at meteorological stations in central and southern Alberta and Saskatchewan, 1917–1935

Source of variation	Degrees of freedom	January		July	
		Sum of squares	Mean square	Sum of squares	Mean square
Between station means	41	19.5932	.4779**	95.5082	2.3295**
Between annual means	18	73.6029	4.0891**	578.3304	32.1295**
Residual	738	100.0325	.1355	872.1397	1.1818
Total	797	193.2286	—	1545.9783	—

** *Exceeds mean square residual, 1% level of significance.*

The mean squares between stations and between years for both months significantly exceed the mean square residual. Differences between the 19-year means for individual stations account for 10.1% of the total January variance, differences between the 42-station averages for individual years account for 38.1%, and 51.8% is attributable to local variation within years. The corresponding figures for July are 6.2, 37.4 and 56.4%, so that although the intra-annual correlation is statistically quite significant, there remains, nevertheless, in both cases an appreciable amount of residual local variation.

Actually, of course, a certain proportion of the observed differences between both station and year averages would be expected to arise from chance combinations of these random local fluctuations. If the true residual, between year and between station variances are denoted by σ_1^2, σ_2^2, and σ_3^2, then the observed residual mean squares s_1^2 of Table VII are direct statistical estimates of σ_1^2 for January and July, but the observed s_2^2 and s_3^2 should be equated to $(42\sigma_2^2 + \sigma_1^2)$ and $(19\sigma_3^2 + \sigma_1^2)$ respectively. Substituting s_1^2 for σ_1^2, the estimates of σ_2^2 and σ_3^2 deduced are 0.09413 and 0.01802 for January, and 0.7369 and 0.06041 for July, the corresponding standard deviations being 0.31 and 0.13, and 0.86 and 0.25 inches. For both months, therefore, the variation between year averages is about three times as great as that between station averages.

When the foregoing residual variances s_1^2, ascribable to irregular local variation within years, were added to the mean square deviation of the 19-year station averages from the graduation formulas of the preceding section, standard deviations of 0.39 inches for January and 1.12 inches for July were obtained. These, which are respectively three and four times the corresponding values in Table VI, indicate the unsatisfactory agreement to be expected on the average between the actual and graduated values of precipitation in individual years, using the regression coefficients of Table IV.

In view of the considerable local variation already known to prevail (2), it seemed unlikely that more satisfactory results would be obtained in individual years even in a smaller area, and this opinion was substantiated when the point was actually investigated. For this purpose, a series of 32 stations lying between north latitude 49½° and 52½°, and between west longitude 103½° and 108½° was selected. Table VIII lists the names of these, together with their latitude, longitude and altitude, and the total precipitation recorded at each during the months of April, July and October, 1935.

The linear regression coefficients b_1, b_2 and b_3 of the precipitation of these months on latitude, longitude and altitude were determined in the usual way, the Normal Equations of the Least Squares solution being:

$$
\begin{aligned}
73{,}456\, b_1 - 4{,}568\, b_2 - 343{,}419\, b_3 &= 272.96;\ -750.80;\ \ 468.76 \\
-4{,}568\, b_1 + 242{,}662\, b_2 + 483{,}723\, b_3 &= 374.14;\ \ -8.76;\ -125.17 \\
-343{,}419\, b_1 + 483{,}723\, b_2 + 3{,}790{,}454\, b_3 &= 552.89;\ 2602.02;\ -1995.23
\end{aligned}
$$

TABLE VIII

LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS, AND TOTAL MONTHLY PRECIPITATION AS INCHES OF RAIN, 1935

Station	Lat. N. of 49°, min.	Long. W. of 103°, min.	Height above sea level, ft.	Total monthly precipitation, 1935		
				April	July	Oct.
Aneroid	42	258	2443	.75	2.69	.65
Anglia	154	310	1861	1.30	1.44	.86
Assiniboia	39	180	2450	.65	2.81	1.51
Beechy	110	266	2180	1.33	3.34	.64
Caron	88	173	1841	.95	1.35	.89
Chaplin	88	220	2202	.24	1.00	.70
Davidson	136	179	2030	.43	2.28	.88
Dundurn	168	210	1737	.53	2.89	1.54
Ft. Qu'Appelle	107	48	1600	.75	2.82	.55
Francis	67	50	1977	.50	4.26	.96
Gravelbourg	52	213	2297	.42	2.17	.75
Harris	164	273	1896	2.51	1.94	1.25
Humboldt	192	129	1865	.35	.62	1.09
Illerbrun	56	320	2925	.30	4.86	.90
Indian Head	88	40	1924	.65	3.12	.98
Lestock	137	57	2219	1.41	2.00	1.30
Lumsden	99	115	1620	.29	2.96	.29
Maskakee	199	161	1787	1.45	1.42	2.01
Moose Jaw	81	155	1860	.42	2.54	.84
Nokomis	150	120	1718	.57	.71	1.25
Outlook	148	245	1774	.43	2.58	1.04
Pennant	92	314	2346	1.91	1.63	.40
Qu'Appelle	91	56	2147	1.10	2.38	1.43
Regina	87	97	1884	.48	1.68	.87
Saskatoon	195	210	1600	1.89	2.78	2.56
Saskatoon Univ.	188	218	1690	1.65	2.50	2.36
Semans	146	106	1845	.81	1.48	.50
Shaunavon	37	322	3010	.66	2.55	.22
Strasbourg	125	117	1799	.40	1.38	.67
Swift Current	80	285	2440	1.53	3.40	.85
Tugaske	111	196	1986	.55	1.48	.40
Yellow Grass	49	69	1899	1.68	5.09	.97

These gave the values for the unknowns shown in Table IX.

TABLE IX

PARTIAL REGRESSION COEFFICIENTS OF MONTHLY PRECIPITATION (INCHES OF RAIN), 1935, ON LATITUDE (b_1), LONGITUDE (b_2) AND ALTITUDE (b_3) OF METEOROLOGICAL STATIONS

Month	b_1, 1/100's in. per 10′ N.	b_2, 1/100's in. per 10′ W.	b_3, 1/100's in. per 100 ft.
April	7.60 ± 2.88	0.03 ± 1.39	8.31 ± 4.64
July	−13.04 ± 5.29	0.95 ± 2.56	6.14 ± 8.52
October	7.68 ± 2.48	−0.95 ± 1.20	2.90 ± 4.00

The coefficients for latitude alone are of any statistical significance. The multiple correlation coefficients R between precipitation and latitude, longitude and altitude for the three months were found to be 0.48, 0.48 and 0.58

respectively, all very moderate values, and the residual standard deviation of precipitation to be 0.53, 0.97 and 0.46 inches respectively. Thus, the major part of the inter-station variance again remains in the form of residual deviations, and the conclusion with respect to this smaller district must be the same as that previously reached for the larger area, *viz.*, that a given number of meteorological stations would provide a much less complete quantitative specification of precipitation than of air temperature.

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A STUDY OF THE PATHOGENICITY OF THE MEADOW NEMATODE AND ASSOCIATED FUNGUS *CYLINDROCARPON RADICICOLA* WR.[1]

By R. J. Hastings[2] and J. E. Bosher[2]

Abstract

The meadow nematode *Pratylenchus pratensis* has been isolated from root lesions of narcissus, strawberry, apple, cherry, and raspberry in British Columbia. When apparently freed from associated fungi, this species reduced the growth of potato, carrot, red clover, tomato, spinach, and violet seedlings by 50 to 75%, and oat seedlings by less than 4%. In parallel experiments, the commonly associated fungus *Cylindrocarpon radicicola* as a pure culture reduced growth by only 6 to 11%. The inhibition of growth by the fungus and nematode as a mixed culture was usually greater than the sum of the reductions as pure cultures.

The nematodes were separated from associated fungi by planting segments of infested oat roots in plate cultures of powdered peat moistened with a 0.1% solution of malachite green. When the oats planted in this medium had germinated, the nematodes moved from the root segments through the fungicidal medium into the roots of the oat seedlings. These nematode-infested roots proved to be free from the fungi and bacteria naturally associated with the nematode in field infestations.

The adult and all the larval stages of *P. pratensis* proved to be capable of entering the roots of oat seedlings.

Introduction

The occurrence of the meadow nematode (*Pratylenchus pratensis* (de Man 1880) Filipjev 1936) as a root parasite in British Columbia and elsewhere has been reported (1-7), but the pathogenicity of the nematode has never been satisfactorily established. Ark and Thomas (1) compared the growth of apple seedlings in healthy soil and soil inoculated with *P. pratensis*. They found that the nematodes caused considerable stunting, but pointed out that the degree of injury could not be determined with certainty owing to the lack of effectual methods for freeing the nematodes from associated bacteria and other organisms.

No investigator has presented convincing evidence that *P. pratensis* is definitely pathogenic when freed from associated organisms. This species is an obligate parasite which is found in the roots of a wide range of plants and in the soil. When found within plant roots it is nearly always associated with other organisms, therefore infested roots cannot be considered as a satisfactory source of inoculum owing to the presence of these associates.

[1] *Manuscript received March 2, 1938.*
Contribution No. 546 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

[2] *Plant Disease Investigator, Dominion Laboratory of Plant Pathology, Saanichton, British Columbia, Canada.*

Experimental

Hosts and Host Preferences of the Meadow Nematode

Attempts were made to develop *P. pratensis* in pure culture upon artificial media without success, hence roots had to be used as a source of inoculum. The preference of the nematodes for the roots of specific plants was investigated to establish the relative value of the roots as living media in the multiplication of the nematodes, and at the same time to obtain information on the relative susceptibility of different species attacked by the meadow nematode. The average numbers of nematodes that entered the roots of different species are given in Table I.

TABLE I

The average number of meadow nematodes that entered seedlings grown in infested strawberry soil

Host	Age of seedlings, days	Number of nematodes in each seedling
Common vetch	8	52
Red Fife wheat	8	3
Early blue pea	8	53
Banner oats	8	208
Barks barley	8	8
Sunflower	8	25
Japanese millet	8	5
Yellow eye bean	9	37
1000-headed kale	9	86
Red clover	9	14
Earliana tomato	16	12
Carrots	16	12
Timothy grass	24	26

The data revealed that all species tested were susceptible. The large numbers found in oats suggested that *P. pratensis* is especially attracted to the roots of this seedling under the growth conditions of our greenhouse.

The affinity of oat seedlings for the nematode can be used to reveal the field distribution, by planting oats on collected soil samples. The number of nematodes in oat seedlings grown on soil samples from an infested strawberry plantation showed that *P. pratensis* was more abundant under the strawberry plants than midway between the rows.

Fungicidal Aids in Freeing the Meadow Nematode from Associated Organisms

Malachite green was selected as a fungicide to free oat roots infested with *P. pratensis* from associated organisms, because of the high tolerance of both oats and the nematode to this chemical. Newton and Edwards (8) have reported that 0.11% is lethal to yeast and *Bacterium Juglandis*. Munro and Newton (9) found that 0.001% malachite green inhibited the growth of *Fusarium culmorum*, *F. moniliforme*, *F. Solani*, *F. orthoceras*, *Pythium ultimum*, and *Rhizoctonia Solani*. Verona (10) found that a concentration of 0.001% arrested the germination of spores of *Tilletia levis*, *T. caries*, *Ustilago Maydis*, and *U. Tritici*, while Verona and Ceccarelli (11) established that 0.0005% inhibited the growth of *Verticillium Amaranti*, *V. albo-atrum*, *V. Dahliae*, and *V. tracheiphilum*.

Nematode-infested oat root segments were placed in a medium containing 0.4 gm. each of disodium phosphate, magnesium sulphate and potassium nitrate, 40 gm. of sucrose, 20 gm. of agar, and varying amounts of malachite green per litre of distilled water. The survival of bacteria, fungi, and nematodes was studied in these cultures. The results are given in Table II.

TABLE II

THE GERMICIDAL AND FUNGICIDAL EFFICIENCY OF MALACHITE GREEN SHOWN BY THE SURVIVAL OF ORGANISMS IN OAT ROOT INOCULATIONS

Conc. of malachite green, %	Organisms that developed in cultures			Remarks
	Bacteria	Fungi	Nematodes	
0.001	+++	++	++	*Fusarium* sp.; *Cylindrocarpon* sp. and bacteria were found.
0.005	+++	++	+	*Fusarium* sp.; *Cylindrocarpon* sp. and bacteria were found.
0.010	++	±	+	Bacteria only were abundant.
0.050	±	–	+	
0.100	–	–	+	
0.500	–	–	+	

The minimum inhibitory concentrations of malachite green were 0.05% for fungi and 0.1% for bacteria. These values are somewhat higher than those reported by Munro and Newton (9) and Verona and Ceccarelli (10, 11), possibly owing to dye absorption by the root tissue.

Tolerance of Meadow Nematode and Oat Seedlings to Malachite Green

When pieces of nematode-infested roots were placed in the middle of a Petri dish, in a medium of peat saturated with 0.1 to 0.6% malachite green and planted with oats, the nematodes moved from the root inoculum through the fungicidal medium into the roots of the oats when the seeds germinated. These peat cultures were used to establish the tolerance of the seedlings and the nematodes to malachite green. The results are shown in Table III.

TABLE III

THE TOLERANCE OF OATS AND THE MEADOW NEMATODE TO MALACHITE GREEN AS SHOWN BY SEEDLING GROWTH AND THE NUMBER OF NEMATODES THEREIN

Conc. of malachite green, %	Seedling growth			Average number of nematodes in a seedling
	Germination, %	Length of top, cm.	Length of root, cm.	
0.01	100	11	3.0 – 4.5	56
0.05	90	11	3.0 – 4.5	53
0.10	90	11	3.0 – 4.5	53
0.20	90	11	2.5 – 4.0	21
0.40	100	8.5	2.0 – 3.0	9
0.60	70	7.0	0.5 – 1.0	5
0.80	100	6.0	0	0

Oats were tolerant of a concentration of malachite green up to 0.1%, but above this concentration the growth was retarded. When root growth occurred, the nematodes moved from the excised root segments to the roots of the living seedlings. Peat cultures containing 0.1% malachite green proved to be the most satisfactory medium for obtaining nematode-infested oat seedlings free from the organisms usually associated with the meadow nematode.

Pathogenicity Studies

The pathogenicity of the organisms towards various species of plants was measured by the growth reduction of seedlings in inoculated soil compared with those raised on steamed soil.

TABLE IV

The effect of inoculating soil with *P. pratensis* and *C. radicicola* on the growth of various seedlings

Crop	Inoculum	Age of plants, days	No. of plants alive	Average weight, gm.	Relative growth, %	Reduction in growth, %
Irish potato	Check	72	17	0.3127	100	—
	C. radicicola	72	20	0.2788	89.1	10.9
	P. pratensis	72	15	0.1266	40.4	59.6
	C. radicicola and *P. pratensis*	72	13	0.0557	17.8	82.2
Carrot	Check	53	15	0.2170	100	—
	C. radicicola	53	15	0.1926	88.7	11.3
	P. pratensis	53	17	0.0923	42.5	57.3
	C. radicicola and *P. pratensis*	53	13	0.5500	25.3	74.7
Red clover	Check	37	10	0.4530	100	—
	C. radicicola	37	16	0.4771	105.3	—
	P. pratensis	37	11	0.1510	35.1	64.9
	C. radicicola and *P. pratensis*	37	16	0.0706	16.4	83.6
Tomato	Check	30	12	0.6137	100	—
	C. radicicola	30	12	0.5759	92.9	7.1
	P. pratensis	30	6	0.1642	26.7	73.3
	C. radicicola and *P. pratensis*	30	11	0.1818	29.6	70.4
Spinach	Check	10	10	0.2920	100	—
	C. radicicola	10	11	0.2730	93.5	6.5
	P. pratensis	10	7	0.0960	32.8	67.2
	C. radicicola and *P. pratensis*	10	7	0.0860	29.5	70.5
Violet	Check	87	4	1.0875	100	—
	C. radicicola	87	4	1.1142	102.4	—
	P. pratensis	87	4	0.2550	23.4	76.6
	C. radicicola and *P. pratensis*	87	4	0.2125	19.5	81.5
Oats	Check	73	10	2.0730	100	—
	C. radicicola	73	10	1.9090	92.0	8.0
	P. pratensis	73	10	1.9970	96.3	3.7
	C. radicicola and *P. pratensis*	73	10	1.7340	83.7	16.3

P. pratensis cultures, purified by passage through peat to oat seedlings in the presence of 0.1% malachite green, were used as the nematode inoculum. Ten infested seedlings were mixed with the soil in a 4-in. pot.

A culture of *Cylindrocarpon radicicola* was grown on steamed whole barley, and four grains served as the fungus inoculum for each 4-in. pot.

The results of these studies are reported in Table IV.

The experiments showed that *P. pratensis* reduced the growth of potato, carrot, red clover, tomato, spinach, and violet seedlings by 50 to 75%, but oat seedlings by less than 4%. No satisfactory explanation can be offered as to why the pathogenicity of the nematode to oats is not more pronounced. Shortly after germination, more nematodes are found in oat roots than in other common crops, as will be seen by referring to Table I. A possible explanation is that the total nematode population of the soil enters the seminal roots, thus allowing the nodal roots to develop.

C. radicicola reduced the growth of the seedlings by 6 to 11% only.

The two organisms as a mixed culture usually caused more damage than the sum of the damage caused by pure cultures.

The Infective Stage of the Meadow Nematode

The life history of *P. pratensis* is not completely known, and Goodey (12, pp. 111-116) surmised that the first stage larva is the probable infective stage. Our experiments reveal that all stages are capable of entering oat roots. Oat seeds sown in a malachite-green peat medium germinated between the third and fourth days. On the fourth day, when the day-old roots were only 2 cm. long, they were found to contain adult as well as larval specimens of *P. pratensis*. Eggs were found in 3-day-old roots. The ability of adult and other stages to invade roots means that large nematode populations in root tissue may be built up rapidly by mass infection from the soil, and by immediate multiplication within the plant.

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THE NATURE OF BULB NEMATODE (*DITYLENCHUS DIPSACI*) POPULATIONS IN "SUPREME", "PRINCE ALBERT", AND "IMPERATOR" IRIS BULBS, AND THEIR CONTROL BY THERMAL TREATMENT[1]

By R. J. Hastings[2] and J. E. Bosher[2]

Abstract

"Supreme" and "Prince Albert", representing a Dutch *tingitana* hybrid and an English iris, are much more susceptible to nematode infestation than "Imperator", a Dutch iris, as judged by the number of nematodes per unit volume of invaded tissue, *viz.*: 596, 108, and 13 respectively. Infestation in "Prince Albert" tends to be confined to the basal plate. Rapid multiplication of the nematodes within the bulb tissue occurs in "Supreme" and "Prince Albert." On the other hand, little multiplication occurs within "Imperator" bulb tissue.

The populations within iris bulb tissue consist largely of young larvae in contrast with a high pre-adult population in narcissi. The low population of the heat-resistant pre-adults accounts for the fact that the nematode population in "Supreme" bulbs can be destroyed by a 60-minute immersion at 110° F., whereas a three-hour immersion is required to destroy the nematodes in narcissi.

Introduction

The symptoms caused by the bulb nematode (*Ditylenchus dipsaci* (Kühn) Filipjev 1936) in iris bulbs differ with species and variety. Steiner and Buhrer (1) reported nematode infestations in more than 22 varieties of irises including Dutch *(I. xiphium hybridum)*, English *(I. xiphioides)*, Spanish *(I. xiphium)* and Tangerian *(I. tingitana)* irises. They considered that the symptoms were the same or very similar in all four species, and that five to six months is sufficient time for an infestation to kill a bulb.

Our observations reveal that bulbs of the *tingitana* hybrid "Supreme" are often killed in a few months, while under the same conditions those of the Dutch iris "Imperator" are little damaged. Bulbs of the English iris "Prince Albert" may have their basal connective tissue completely invaded and destroyed without any evidence of attack on the bulb scales.

Experimental

A study of the progress of nematode infestation in iris bulbs and of the nematode populations in invaded tissue indicates that the nematodes first enter and become established in the basal plate of "Supreme". Rapid multiplication occurs in the basal plate tissue; from this region they penetrate into the bulb scales, and continue to advance and multiply until the whole bulb becomes spongy in texture, gray in color, and shrivelled in appearance. Among infested lots of "Supreme" bulbs, mummified specimens and others with large gray areas are commonly found at planting time.

The nematodes also enter the basal plate of "Imperator" and eventually work their way into the bulb scales, but little multiplication occurs in either

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[2] *Plant Disease Investigator, Dominion Laboratory of Plant Pathology, Saanichton, B.C.*

basal tissue or bulb scale. Hence the nematode populations are generally small and cause only a few streak lesions.

The nematodes entering the basal plate of "Prince Albert" breed and deposit many eggs. Under storage conditions the basal tissue becomes too dry for normal development, hence the populations in this variety rarely reach the size found in "Supreme". The basal tissue of "Prince Albert" is very large and there is seldom time for the infestation to progress beyond the basal plate. The damage sustained is usually confined to the basal tissue.

Since the mother bulb of the iris disappears when the new bulbs develop, a nematode infestation is the result of infestation during the current season. The consistently large nematode populations of "Supreme" and "Prince Albert" and small populations of "Imperator" are probably due to the relative ability of the respective tissue to support a nematode population. Kreis (3) studied the populations of *D. dipsaci* infesting sweet potatoes, and reported that the population in the outer 1.5 mm. of the tuber was more than 15 times greater than that of the inner layers. He suggested that the distribution of richer and more suitable food material near the skin is the probable reason. The appearance of a nematode infestation in the three classes of bulbs investigated by the writers is shown in Fig. 1.

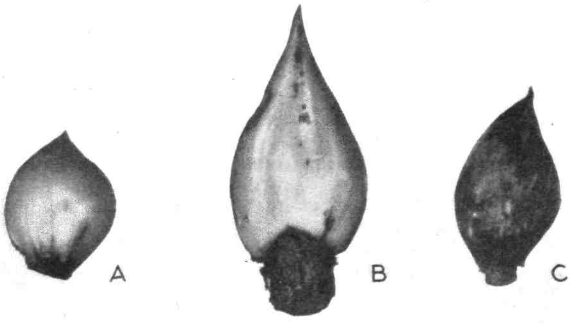

FIG. 1. *The injury caused by D. dipsaci to iris bulbs. A. Small streak lesions in fleshy scale of Dutch iris var. "Imperator". B. Sponginess and discoloration of basal tissue of English iris var. "Prince Albert". C. Sponginess and discoloration of entire outer fleshy scale of tingitana hybrid, var. "Supreme".*

A quantitative study of the nematode populations in the three classes of bulbs was made by suspending approximately 5 cu. mm. of macerated bulb tissue from each in 20 cc. of tap water, followed by a count of the nematodes in 10 microscopic fields of approximately 2.6 sq. mm. area. Although

"Supreme", "Prince Albert", and "Imperator" represent three bulb classes, the analysis of their nematode populations as given in Table I may represent varietal rather than class distinctions in susceptibility.

TABLE I

COUNTS OF NEMATODES IN SUSPENSIONS OF IRIS TISSUE

"Imperator"				"Supreme"				"Prince Albert"			
Adult	Pre-adult	Larvae	Eggs	Adult	Pre-adult	Larvae	Eggs	Adult	Pre-adult	Larvae	Eggs
1	0	0	0	0	1	68	3	2	0	17	18
0	1	1	0	0	0	40	2	2	2	11	31
0	0	1	0	2	1	65	1	3	0	15	45
0	0	1	0	0	0	53	2	1	1	4	16
0	1	2	0	0	0	58	3	2	1	5	34
1	0	1	0	1	1	48	0	2	4	3	19
0	0	0	0	1	0	59	4	2	0	4	45
0	0	0	0	1	1	69	2	1	2	5	45
0	1	0	0	1	0	66	1	1	3	5	46
1	0	1	0	2	0	48	1	3	3	4	42
3	3	7	0	8	4	584	19	19	16	73	341

The resistance of "Imperator" tissue to the bulb nematode is suggested by the small numbers in the lesions, a total of 13 in contrast with 596 in "Supreme", and 108 in "Prince Albert". The susceptibility of the last two is further emphasized by the fact that the invaded zone was many times greater than in the first-named. Infested "Supreme" and "Prince Albert" bulbs are a much greater menace as sources of inoculum than "Imperator", for they usually carry greater nematode populations.

In contrast with the large number of pre-adult nematodes in narcissus bulbs, the populations in iris consist largely of eggs and immature larvae. Since the pre-adult larvae are more resistant to heat than the eggs and immature larvae (2) it follows that iris need not be subjected to the prolonged heat treatment prescribed for narcissi. While our investigations show that immersion for three hours at 110–112° F., preceded by a 12-hour immersion in water at room temperature, is necessary for narcissi, a one-hour immersion at the same temperature effectively destroys the nematodes in iris bulbs when the treatment is preceded by a 2.5-hour immersion in water at room temperature.

"Supreme" bulbs were immersed in water at 110° F. for varying periods, and the treated tissue was macerated and suspended in water for microscopic examination.

More than 95% of the nematodes in the control were motile; but less than 0.1% were motile after the bulbs were immersed in hot water for 45 min., and none survived 60 min. A one-hour immersion of infested iris bulbs in water at 110° F. is apparently sufficient to destroy the nematode popula-

tions in iris, even when the bulbs are not pre-soaked to increase the susceptibility of the populations to heat.

TABLE II

THE MOTILITY OF BULB NEMATODES IN INFESTED "SUPREME" BULBS AFTER TREATMENT IN WATER AT 110° F. FOR VARYING PERIODS

Class of nematodes	Time of immersion in minutes								
	0	30	45	60	75	90	105	120	150
Adults	++++	+	±	–	–	–	–	–	–
Pre-adults	++++	+	±	–	–	–	–	–	–
Larvae	++++	+	–	–	–	–	–	–	–

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OBSERVATIONS ON THE STUDY OF VARIETAL DIFFERENCES IN THE MALTING QUALITY OF BARLEY. PART II[1]

By J. Ansel Anderson[2] and Henry R. Sallans[2]

Abstract

Samples of O.A.C. 21 and Wisconsin 38 barley from two stations were germinated at 56° and 50° F. with 44.5% moisture, and with 44.5 and 42.5% moisture at 53° F. Aliquots were kilned and analyzed after 3, 5, 7, 9, 11 and 13 days. Data for extract, diastatic power, and permanently soluble nitrogen, as percentage of wort solids, were plotted against time. Both varieties responded in almost exactly the same manner to changes in temperature and moisture. *Values for O.A.C. 21 were consistently higher, but paired curves, representing* samples of both varieties from the same station, became closer with increasing time, owing largely to overmodification of the O.A.C. 21. A real difference in malting quality between these two varieties, greater than the differential effect of *malting method on them, is therefore indicated.*

In the first paper of this series (3) it was pointed out that three main factors affect the determination of varietal differences in the malting quality of barley. These factors are: (i) the precision of the malting test; (ii) the differential effect of environment on varieties and (iii) the differential effect of malting method on varieties.

Investigation (2, 3, 5) has convinced us that the first of these is of minor importance. A considerable body of data on the effect of the second factor is rapidly being accumulated in several countries (3, 4, 6–9, 11, 12). This shows that the differential effect of environment on varieties is definitely a major complicating factor in studies of malting quality. In consequence, broad generalizations are justified only with respect to differences between certain varieties, the relative performance of others being considerably affected by the prevailing environmental conditions.

The third factor, the differential effect of malting method on varieties, has received little direct study although information concerning it is available as a side-product of two recent investigations. Berglund (6) had samples of several varieties malted in three commercial plants. It seems safe to assume that all plants used similar methods, designed for malting the two-rowed barleys under test to good advantage, and thus no very considerable differential effect of method on varieties would be expected. The data show that the effect was very small. A similar limitation existed in our own study (3) in which laboratory and stocking methods, which closely simulated commercial practice, were used. In these circumstances, it is not surprising that the available data suggest that the differential effect of malting method on varieties is by no means as large as the differential effect of environment.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Published as Paper No. 134 of the Associate Committee on Grain Research of the National Research Council of Canada and the Dominion Department of Agriculture. Presented by R. Newton, F.R.S.C., to the Royal Society of Canada, May, 1938.
[2] *Biochemist, National Research Laboratories, Ottawa.*

It is difficult to elucidate the effects of malting methods on different varieties when comparisons are confined to analyses of samples of the finished malt. It appears, however, that the difficulty can be largely overcome by withdrawing and analyzing aliquots of the samples at intervals during the germination period. The effects of malting methods can then be represented by curves rather than by single points and interpretation of results is facilitated. Thunaeus and Schröderheim (12) have used this technique for comparing different varieties under the same malting conditions, while Piratzky and Rehberg (10) and earlier investigators have used it in studying the effects of different malting conditions on samples of one or more commercial barleys.

In laboratory malting studies, the capacity of the equipment sets certain limits to the number of samples which can be studied to advantage by this method. Moreover, its use creates a relatively large increase in the work required for the study of each sample. For these reasons the present investigation was limited to samples of two varieties of barley from two stations.

One of these varieties, O.A.C. 21, represents the Manchurian class of closely related six-rowed, rough-awned varieties, which has been found satisfactory for malting purposes in Canada. The other, Wisconsin Pedigree 38, represents a comparatively new class of smooth-awned, six-rowed varieties, having Lion as one of the original parents. Routine laboratory malting tests (4, 8, and unpublished results) have shown that these smooth-awned barleys are characterized by low extract yield and enzymatic activity. Canadian maltsters do not like them.

In the investigation reported in this paper, an attempt was made to determine whether the differences between O.A.C. 21 and Wisconsin 38, which are demonstrated under standard conditions in the routine test, would persist when the varieties were compared over a range of malting conditions. Although definite conclusions can hardly be drawn from the study of only two samples of each, the data appear to be of sufficient interest to merit publication. Moreover, they provide information that can be used to advantage in interpreting the results of further studies in this series, in which only two germination times are used.

Materials and Methods

Comparable samples of O.A.C. 21 and Wisconsin Pedigree 38 barley were obtained from Indian Head, Saskatchewan, and from Nappan, Nova Scotia. The samples had the following nitrogen contents:— O.A.C. 21, from Indian Head, 2.51%, and from Nappan, 1.39%; Wisconsin 38, from Indian Head, 2.77%, and from Nappan, 1.43%.

Portions representing 1 kg. of dry matter, distributed equally among four cages, were malted in equipment at the National Research Laboratories (1) under the following germination conditions:— 56° F. (chamber temperature) and 44.5% moisture (in steeped barley), 50° F. and 44.5% moisture; 44.5% moisture and 53° F., and 42.5% moisture and 53° F. Aliquots representing one-sixth of the original were removed and kilned under uniform conditions after 3, 5, 7, 9, 11 and 13 days in the germination chamber. The samples of

barley grew continuously and steadily during the whole 13 days, and it was not necessary to sprinkle any of them. Duplicate maltings were not made since it appeared that the smoothness of the curves would provide an adequate estimate of precision. Analyses were made by methods previously described (2).

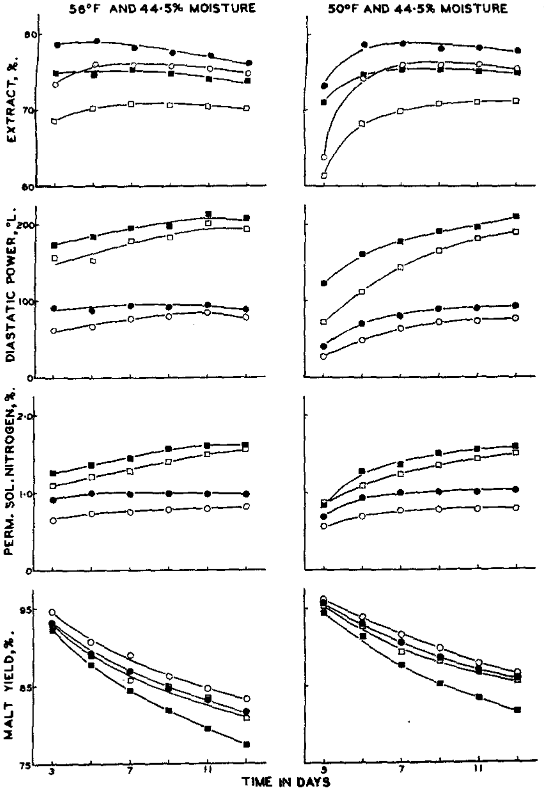

FIG. 1. *Effect of temperature and germination time on the quality of malts made from O.A.C. 21 and Wisconsin 38.* ● *O.A.C. 21, Nappan;* ○ *Wisconsin 38, Nappan;* ■ *O.A.C. 21, Indian Head;* □ *Wisconsin 38, Indian Head.*

Results and Discussion

The data for extract, diastatic power, permanently soluble nitrogen as percentage of wort solids, and malt yield, the four main qualities in which the varieties differed, are presented in Figs. 1 and 2 as curves in which each quality is plotted against germination time. The four rows of graphs

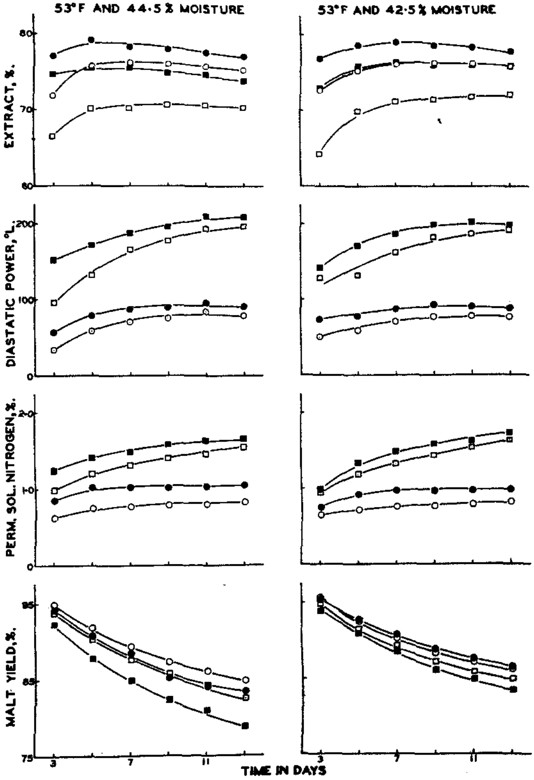

FIG. 2. *Effect of moisture content and germination time on the quality of malts made from O.A.C. 21 and Wisconsin 38.* ● *O.A.C. 21, Nappan;* ○ *Wisconsin 38, Nappan;* ■ *O.A.C. 21, Indian Head;* □ *Wisconsin 38, Indian Head.*

represent the four malt qualities and the four columns of graphs represent the four sets of malting conditions. The individual graphs may best be considered as two pairs of curves, each pair representing samples of the two varieties from one station.

The curves for malt yield are of interest mainly because they show that the samples grew continuously throughout the whole germination period. They also show that Wisconsin 38 grew more slowly than O.A.C. 21. The curves for the former are above the corresponding ones for the latter except for the samples from Nappan germinated with 42.5% moisture. The exception probably indicates that O.A.C. 21 is less tolerant of understeeping than Wisconsin 38.

It is apparent that for extract, diastatic power and soluble nitrogen, the curves for Wisconsin 38 fall below the corresponding ones for O.A.C. 21 and that the curves become closer with increasing time. A differential response of varieties to germination time is thus demonstrated, longer times favoring Wisconsin 38. The effect with respect to extract is attributed largely to over-modification of the O.A.C. 21, which undoubtedly took place towards the end of the germination period. This was to be expected, since this variety can usually be properly modified in about six days under conditions representing approximately the mean of those used in this investigation. When a normal degree of modification is exceeded, the decrease in soluble dry matter through respiration and root loss begins to overtake the increase resulting from the activity of the enzymes, with the result that the curves begin to flatten out, pass the maximum, and then start to fall.

The effect on the two varieties of changing the germination temperature can be observed by comparing curves in the first column of graphs in Fig. 1 with corresponding curves in the second column of graphs, and the effect of changing the moisture content can be observed by making similar comparisons between curves shown in Fig. 2. It is apparent that both responded in almost exactly the same manner to changes in these two conditions of germination. A small differential effect exists, but it is much less than the differential effect of germination time on varieties. This was probably to be expected since the range of times used was greater than the range of either temperature or moisture content.

The general effect of changing the germination conditions is illustrated more clearly by the curves in Fig. 3 which were plotted from data representing the means for all four samples. It is apparent that extract, diastatic power, and permanently soluble nitrogen all increased more rapidly at the higher temperature but that the maximum values obtained were little affected. At the lower moisture content, extract developed more slowly but attained a higher maximum value. In view of the experimental errors of the investigation, it seems doubtful whether any significance should be attached to the small difference between the curves for diastatic power. The effect of moisture content on soluble nitrogen was also small, though there is an indication that development was less rapid but attained a higher maximum value at the lower moisture content.

Piratzky and Rehberg (10), and earlier continental workers cited by them, found that, in general, lower germination temperatures produced higher extract yields and higher enzymatic activity. Our study fails to support these findings. However, it seems unwise to attach any special significance to this lack of agreement. The six-rowed barleys used in our study were

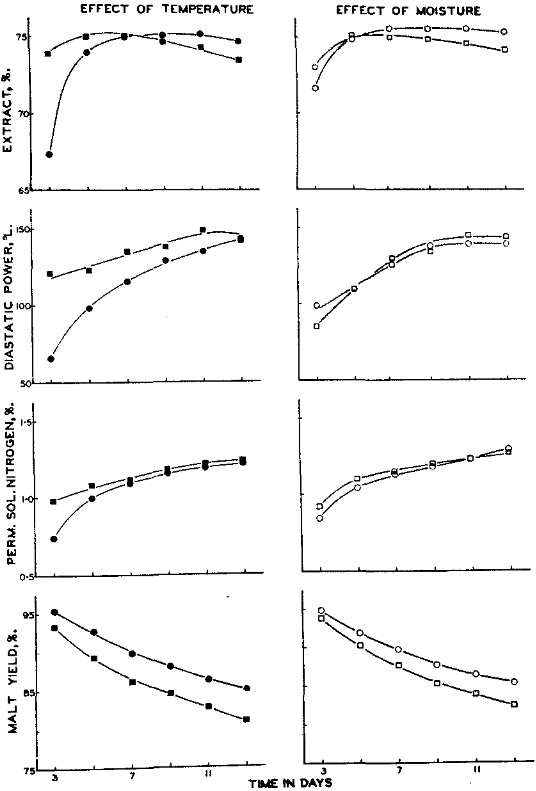

FIG. 3. *Average effect of temperature, moisture and germination time.* ■ *56° F. and 44.5% moisture.* □*44.5% moisture and 53° F.* ●*50° F. and 44.5% moisture.* ○*42.5% moisture and 53° F.*

high in nitrogen content and differ considerably in their malting qualities from the two-rowed barleys grown on the continent. Moreover, the temperatures used differed both in range and level, higher temperatures being used by continental workers in studying barleys of lower nitrogen content.

Although definite conclusions cannot be drawn from the results of a study of only two sets of samples, the data strongly suggest that there are real differences in the malting qualities of the two varieties studied, and that these differences are large in comparison with the effect on them of changing the malting conditions. It appears that the deficiencies of Wisconsin 38 cannot be overcome by such modifications of the malting method as may be considered economically practicable. With respect to extract, the evidence presented is quite clear cut. Even when the germination period is doubled, a procedure that would certainly be very uneconomical, Wisconsin 38 fails to produce as high an extract as the corresponding sample of O.A.C. 21. With respect to diastatic power and wort nitrogen, the statement must be modified, since with the Indian Head samples, increasing the germination period for Wisconsin 38 enables it to attain values as high as those given by O.A.C. 21 in a normal six-day germination period. However, when both varieties are malted in the same manner, values for O.A.C. 21 are consistently higher than corresponding values for Wisconsin 38.

We are inclined to believe that these conclusions, based mainly on the effect of increasing the germination period, would apply equally well to changes in other conditions of malting, since it seems reasonable to suppose that doubling the germination time produced greater effects than could be obtained by any reasonable modifications of steeping, sprinkling, aeration or temperature conditions.

In spite of these conclusions, it must be admitted that the curves show that the differential effect of malting conditions on varieties is appreciable, and it appears that this might well be a limiting source of error in the study of certain pairs of varieties. In these circumstances, it seems probable that the routine malting test, involving the use of one standard set of conditions, will find its greatest use in the preliminary separation of new varieties into those which are about equal in malting quality to the standard variety and those which are definitely inferior to it. More thorough investigations of the type reported in this paper will then be required for the final study of any variety which malting and agronomic data have shown to be really promising.

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THE EFFECT ON WHEAT QUALITY OF LONG EXPOSURE TO CARBON TETRACHLORIDE[1]

By R. K. Larmour[2] and H. N. Bergsteinsson[3]

Abstract

Wheat samples (5 lb.) of 12, 18 and 24% moisture content were stored at 21° C. for periods varying from 4 to 40 weeks with dosages of carbon tetrachloride from 1 to 20 cc. At 12% moisture content the wheat was not damaged by either storage or carbon tetrachloride. At 18% moisture content, samples with 10 and 20 cc. dosages of carbon tetrachloride were undamaged after 10 weeks' storage except for a slight sour odor which disappeared on drying. After 20 weeks' storage there was definite evidence of damage. Samples of 24% moisture content kept well for 4 weeks without any carbon tetrachloride, but those having the higher dosages were damaged. With longer storage all samples at 24% moisture content, no matter how treated, underwent spoilage.

Introduction

In connection with a program of investigations on the prevention of damp wheat spoilage, Larmour, Clayton and Wrenshall (2) reported that exposure of damp wheat for 25 days to sufficient carbon tetrachloride to prevent heating caused no deterioration of baking quality attributable to the chemical. They used wheat at 24% moisture content and dosages of carbon tetrachloride varying from 1 to 12 cc. per 5-lb. sample of wheat. It was observed, however, that storage at this moisture content without any carbon tetrachloride caused a marked decrease in resistance to high dosages of flour improvers such as potassium bromate, and combinations of potassium bromate, diastatic malt, and ammonium phosphate. This was not noticeable with the simple baking formula and with formulas involving dosages of potassium bromate alone up to 0.003%. Larmour (1), in an earlier study, found that storage at moisture contents up to 22% did not affect the baking quality except in cases where the wheat became moldy. As only the simple and bromate formulas were used in this earlier study, it appears probable that failure to discover damage may be attributed to the inadequacy of the baking tests used at that time. In 1934, Swanson (3) reported that damp wheat stored with the fungicide Ceresan (2% ethyl mercuric chloride in 98% inert dry material) showed no apparent damage to baking strength as a result of 4 weeks' storage, but after 13 to 16 weeks' storage there was a marked decrease in loaf volume. This indicated that our storage experiment might not have been continued long enough to make a rigorous test of the action of carbon tetrachloride on damp wheat. For these various reasons it seemed advisable to repeat the work, using both treated and untreated samples, and longer storage periods.

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[2] *Professor of Chemistry, University of Saskatchewan.*

[3] *Research Assistant, National Research Council.*

Material and Treatment

The wheat used was a uniform four-bushel lot of Marquis, grown at Saskatoon in 1934. When received it had a moisture content of 11.3%, protein content of 17.5%, and was graded No. 1 Northern. The samples were stored in tin cans 7 in. high by 6 in. in diameter, fitted with slip covers which were heavily paraffined at the joints. Five pounds of wheat practically fill these cans. They were stored in a sub-basement room which is kept at 21° C. continuously by thermostatic control. The scheme of the experiment is given in Table I.

TABLE I

PLAN OF THE STORAGE EXPERIMENT

Dosage of CCl_4, cc.	Time of storage, weeks	Sample No. — Initial *moisture of stored* wheat: 12%	18%	24%
None	4	1	2	3
None	10	13	14	15
None	20	25	26	27
None	40	37	38	39
1	4	4	5	6
1	10	16	17	18
1	20	28	29	30
1	40	40	41	42
10	4	7	8	9
10	10	19	20	21
10	20	31	32	33
10	40	43	44	45
20	4	10	11	12
20	10	22	23	24
20	20	34	35	36
20	40	46	47	48

As the appropriate cans were opened after each storage period, the samples were carefully examined for odor and appearance; samples were taken for moisture determinations, and those with 18% and 24% moisture were spread on screen-bottomed trays to dry. After drying to about 13% or lower, they were tempered and milled in the usual way, and baked after the flour had aged for one month. Two baking formulas were used:— (i) the standard malt-bromate-phosphate formula (namely, NaCl 1%, sugar 2.5%, yeast 3.0%, 200° Lintner malt 0.3%, $KBrO_3$ 0.001%, and $NH_4H_2PO_4$ 0.1%) and (ii) a high intensity formula with all ingredients the same as (i) except yeast 5% and sugar 6%. Mixing procedure, fermentation times and temperatures were as prescribed for the A.A.C.C. standard baking test.

Discussion of the Results

The baking data, together with observations on the condition of the wheat when taken from storage, are given in Tables II, III and IV. They are grouped according to moisture content of the samples.

As was expected, there was no appreciable change in wheat at 12% moisture with any of the dosages of carbon tetrachloride used.

Wheat stored at 18% moisture for 10 weeks showed no evidence of deterioration of baking quality. The check sample and the sample with 1 cc. carbon tetrachloride were slightly musty in smell; those with higher dosages were slightly sour but not musty. These odors of the wheat samples could not

be detected in the flours or in the bread. Storage for 20 weeks and longer resulted in damage to baking quality even with the smallest dosages of carbon tetrachloride.

The samples at 24% moisture, after four weeks' storage, gave no evidences of mustiness. They all had a slight sour odor which was not carried over to the flour or bread. The baking data, however, revealed that, while the check sample was undamaged, the treated ones showed distinct evidence of damage to quality. The extent of the damage was slight in the sample stored with 1 cc. of carbon tetrachloride but quite severe in the two other samples. All other samples in the 24% moisture-content series were damaged. The extent of the damage to quality increased with time, and in all cases those treated with the 10 and 20 cc. dosages deteriorated more than either the untreated check sample or the sample treated with 1 cc. of carbon tetrachloride. It is evident, therefore, that carbon tetrachloride has a deleterious effect on wheat at 24% moisture content.

Attention should be directed to Sample 15, Table IV. This sample (which was untreated) gave no evidence of mustiness, only a very slight sour odor, and was of excellent color and appearance, but it had undergone pronounced deterioration in baking quality. This damage to baking quality must be attributed to changes induced by the high moisture content. The exceptionally fine color of these high-moisture samples was in striking contrast to the pronounced bleached appearance of the samples stored at 18% moisture. No explanation of this has been found yet.

TABLE II

DATA ON WHEAT STORED AT 12% MOISTURE CONTENT

Sample No.	Time of storage, weeks	Dosage of CCl_4, cc.	Moisture after storage, %	Malt-bromate-phosphate Formula (1)		High-speed Formula (2)		Remarks on condition of wheat after storage	
				Loaf vol., cc.	Baking score	Loaf vol., cc.	Baking score	Color of wheat	Odor of CCl_4
1	4	0	12.1	960	159	980	166	No change	
4	4	1	12.2	1035	174	965	163	No change	Slight
7	4	10	12.2	1005	168	1050	180	No change	Strong
10	4	20	12.4	1040	176	950	161	No change	Very strong
13	10	0	12.2	1075	184	1150	198	No change	
16	10	1	12.3	1145	199	1125	192	No change	Slight
19	10	10	12.5	1075	184	1150	197	No change	Strong
22	10	20	12.4	1115	192	1125	192	No change	Strong
25	20	0	12.3	1035	174	1040	184	No change	
28	20	1	12.6	1085	184	1060	190	No change	None
31	20	10	12.5	1095	187	1055	186	No change	Strong
34	20	20	12.2	1008	170	1045	184	No change	None
37	40	0	12.2	1080	191			No change	
40	40	1	12.3	1150	204			No change	None
43	40	10	12.2	1090	192			No change	Slight
46	40	20	12.2	940	163			No change	Slight

TABLE III

DATA ON WHEAT STORED AT 18% MOISTURE CONTENT

Sample No.	Time of storage, weeks	Dosage of CCl_4, cc.	Moisture after storage, %	Malt-bromate-phosphate Formula (1)		High-speed Formula (2)		Remarks on condition of wheat after storage			
				Loaf vol., cc.	Baking score	Loaf vol., cc.	Baking score	Color of wheat	Odor of CCl_4	Musty odor	Sour odor
2	4	0	18.2	1000	167	910	152	Slightly bleached	–	Slight	–
5	4	1	18.4	1025	174	955	161	Slightly bleached	Slight	Slight	–
8	4	10	17.9	905	148	960	162	Slightly bleached	Strong	Nil	Nil
11	4	20	17.9	935	157	900	150	Slightly bleached	Strong	Nil	Nil
14	10	0	18.6	1025	175	1010	170	Quite bleached	–	Slight	Nil
17	10	1	18.6	1075	184	1035	174	Quite bleached	Nil	Slight	Nil
20	10	10	18.2	1010	170	1070	180	Quite bleached	Slight	Nil	Slight
23	10	20	17.8	1030	175	1005	168	Slightly bleached	Slight	Nil	Slight
26	20	0	18.2	905	149	880	151	Bleached	–	Slight	Very slight
29	20	1	18.2	940	157	890	152	Bleached	Nil	Slight	Nil
32	20	10	17.8	932	155	835	139	Bleached	Nil	Nil	Slight
35	20	20	17.0	885	145	873	147	Bleached	Nil	Slight	Slight
38	40	0	18.7	820	125			Bleached	–	Slight	–
41	40	1	19.1	895	146			Bleached	Nil	Marked	Marked
44	40	10	18.4	870	147			Slightly bleached	Slight	Slight	Marked
47	40	20	18.3	785	126			Bleached	Nil	Slight	Slight

TABLE IV

DATA ON WHEAT STORED AT 24% MOISTURE CONTENT

Sample No.	Time of storage, weeks	Dosage of CCl_4, cc.	Moisture after storage, %	Malt-bromate-phosphate Formula (1)		High-speed Formula (2)		Remarks on condition of wheat after storage			
				Loaf vol., cc.	Baking score	Loaf vol., cc.	Baking score	Color of wheat	Odor of CCl_4	Musty odor	Sour odor
3	4	0	24.1	995	166	945	156	Slightly bleached	–	Nil	Slight
6	4	1	23.9	950	157	870	141	Slightly bleached	Nil	Nil	Slight
9	4	10	24.0	795	126	710	110	Good	Slight	Nil	Slight
12	4	20	23.7	765	126	620	91	Good	Marked	Nil	Slight
15	10	0	24.4	800	120	600	75	Good	–	Nil	Slight
18	10	1	24.4	860	131	750	111	Good	Very slight	Very slight	Slight
21	10	10	24.1	625	79	615	82	Good	Slight	Nil	Slight
24	10	20	24.5	575	68	520	55	Good	Marked	Nil	Slight
27	20	0	25.0	648	68	578	49	Slightly bleached	–	Slight	Marked
30	20	1	24.8	840	104	775	89	Slightly bleached	Nil	Marked	Marked
33	20	10	23.8	470	25	440	15	Excellent	Nil	Slight	Marked
36	20	20	24.3	415	13	435	14	Excellent	Marked	Slight	Marked
39	40	0	25.4	440	15			Bleached	–	Very musty	Very sour
42	40	1	25.0	425	16			Slightly bleached	Nil	Slight	Marked
45	40	10	24.7	360	2			Excellent	Nil	Slight	Marked
48	40	20	25.6	357	−2			Excellent	Slight	–	Marked

The baking data given in Tables II, III and IV were obtained at different times and therefore are subject to rather large errors, if one wishes to compare the results of the various storage periods. In order to get more comparable data, all the samples of flour from the preceding series were rebaked by Formula 1 at the time of baking the 40-week series. The results are given in Table V. There is, of course, another source of variation in this series of values, namely, the aging of the earlier-milled flours. There is no way of knowing whether the keeping quality of some of the flour samples had been damaged by the treatment of the wheat, but if one compares these values with those obtained in the earlier bakings, there is found little evidence of deterioration due to storage as flour. From the data of the final baking in Table V, the following conclusions seem to be warranted: (i) Dry wheat suffers

TABLE V

LOAF VOLUMES OBTAINED IN THE FINAL BAKING OF THE FLOURS BY THE MALT-BROMATE-PHOSPHATE FORMULA

Dosage of CCl_4, cc.	Time of storage as wheat, weeks	Time of storage as flour, weeks	Loaf volumes			Loaf volume — first baking — after flour was stored 4 weeks		
			12%	18%	24%	12%	18%	24%
0	4	40	1040	920	947	960	1000	995
1	4	40	1095	1065	975	1035	1025	950
10	4	40	985	1070	780	1005	905	795
20	4	40	1062	965	790	1040	935	765
0	10	34	985	915	815	1075	1025	800
1	10	34	1080	942	886	1145	1075	800
10	10	34	1025	995	600	1075	1010	625
20	10	34	1105	967	490	1115	1030	575
0	20	24	1110	910	672	1035	905	648
1	20	24	1150	910	810	1085	940	840
10	20	24	1015	945	380	1095	932	470
20	20	24	1040	930	380	1008	885	415
0	40	4	1080	820	440			
1	40	4	1150	895	425			
10	40	4	1090	870	360			
20	40	4	940	785	357			

no damage from exposure to carbon tetrachloride over a period of 40 weeks. (ii) Wheat at 18% moisture content shows some deterioration as a result of storage for 40 weeks. This cannot be attributed to the effect of carbon tetrachloride, but rather to the mustiness and sourness developed. (iii) Wheat at 24% moisture content suffered no damage when stored 4 weeks without carbon tetrachloride or any other fungicide and only slight damage when stored with 1 cc., but with dosages of 10 and 20 cc. there was marked evidence of damage attributable to the carbon tetrachloride. After 10 weeks' storage, the check sample, although showing no marked physical damage, gave distinct evidence of deterioration. This could be attributed only to the high moisture

content. The effect of carbon tetrachloride was more pronounced than after 4 weeks' storage. After 20 weeks' storage all samples were heavily damaged. (iv) The baking behavior of some of the more severely treated samples indicates that the high-speed baking formula may prove useful in accentuating quality differences where damage is likely to have occurred.

These results are similar to Swanson's (3) conclusions concerning the action of Ceresan and indicate that with very damp wheat, carbon tetrachloride should be used only for relatively short periods. The four-week period seems safe enough with sound wheat at moisture contents as high as 24%; this should generally be sufficient time to move the grain into a drier, where the removal of moisture would prevent any damage from carbon tetrachloride remaining in the grain.

It is evident that in this study there are two effects which require further investigation. The more important of these is the apparent deterioration occurring when wheat is kept at a high moisture content. In the experiment reported the samples were stored at 21° C., and even at that relatively high temperature those at 24% moisture content were practically sound after 10 weeks' storage in sealed containers. At lower temperatures the storage periods might be extended without causing spoilage. All properties of the wheat that can be estimated with fair accuracy should be investigated in order to find, if possible, what changes occur under these conditions.

The other problem is concerned with the rapid disappearance of carbon tetrachloride in wet grain and the accompanying production of sourness, which may be the result of catalytic hydrolysis, with the production of hydrochloric acid and carbon dioxide. This can be tested readily and should be of academic, if not of practical, interest.

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OBSERVATIONS ON THE STUDY OF VARIETAL DIFFERENCES IN THE MALTING QUALITY OF BARLEY. PART III[1]

By J. Ansel Anderson[2] and W. O. S. Meredith[3]

Abstract

Samples of eight barley varieties grown at six widely separated points in Canada were malted in duplicate under standard conditions in laboratory equipment. After six days in the germinator, half of each sample was removed and kilned. The remaining halves were grown two days longer before kilning. The relative positions of the varieties with respect to extract, diastatic power, and permanently soluble nitrogen, were changed by the additional two days' growth, but the changes were generally small by comparison with the spreads between varieties and the greater changes in their relative positions when grown at different stations. It is concluded that the differential effect of malting method is an appreciable source of error in the interpretation of the results of routine malting tests, but that the limiting factor in studies of the comparative malting qualities of varieties is the differential effect of environment on them.

In Part II of these studies (2) the differential effect of malting method on barley varieties was investigated, using six germination times and four sets of malting conditions, but samples of only two varieties from two stations. The present study was undertaken concurrently and is of the same type. In a sense, it is complementary to the preceding one since samples of eight barley varieties from six stations were used, but the malting conditions represented germination for six and eight days only. These limitations were imposed because the study was carried out, as an extension of an ordinary variety trial, in the laboratory in which routine malting tests are made for Canadian plant breeders. The six-day germination period represented the standard conditions required for the original trial. The eight-day period was chosen as representing a reasonable change in malting procedure and one which could be used with little disruption of the routine of the laboratory.

Materials and Methods

Samples of the six-rowed, rough-awned varieties, O.A.C. 21, Olli, Peatland, and Pontiac; the six-rowed, smooth-awned varieties, Nobarb, Regal, and Velvet; and the two-rowed, smooth-awned variety, Rex; were obtained from the following stations: Beaverlodge and Edmonton, Alberta; Melfort, Saskatchewan; Brandon and Gilbert Plains, Manitoba; and Ste. Anne de la Pocatière, Quebec. The varieties differ widely in their malting characteristics and represent a good cross-section of the materials submitted for malting tests in Canada.

The barleys were grown in plots of five rod-rows arranged in a modified balanced block with quadruplicate plots for each variety. Marginal effects

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[2] *Biochemist, Division of Biology and Agriculture, National Research Laboratories, Ottawa.*
[3] *Senior Research Assistant, Department of Plant Science, University of Manitoba, Winnipeg.*

were reduced by harvesting only the centre three rows, and samples for malting were obtained by bulking the grain from quadruplicate plots. The eight varieties were selected from a total of twenty-four which were being grown mainly for the study of agronomic characters. It is apparent that under these conditions field errors resulting from soil heterogeneity will probably be somewhat greater than they would have been if the eight varieties used in this study had been grown in smaller blocks.

The six-day malts were made and analyzed at the University of Manitoba, using laboratory malting equipment and methods which have been described previously (1). After the samples had been in the germinator for six days, half of each was removed and kilned. The remaining halves were sprayed with 15 gm. of water and grown for an additional two days before kilning.

Results and Discussion

Since the magnitude of the differential response of the varieties to the change in malting conditions may be expected to depend on the magnitude of the average effect of the change on all varieties, this must be examined first. The required data are given at the top of Table I as means, over all varieties, for six- and eight-day malts. The additional two days in the germination chamber resulted in an average increase of 0.2 in percentage extract, 13° Lintner in diastatic power, 0.17 in permanently soluble nitrogen as percentage of wort solids, and 3.5 in percentage malting loss. It is therefore apparent that considerable growth and modification took place during the extra two days, and that opportunity was provided for varieties which grow and modify slowly to show to better advantage in the eight-day series.

The second line of figures in Table I gives the differences between variety means which can be considered statistically significant (five per cent level) in view of the differential response of varieties to environment, which appears to be a limiting factor in investigations of varietal differences in malting quality. (References on this point were listed in Part II of this series, 2.) It may be assumed that varieties whose means differ by less than the amounts listed will fall in different orders at different stations, and must therefore be considered about equal with respect to the malt quality in question. On the other hand, when the means for two varieties differ by the required amount, the odds are 19 to 1 that a real difference between the varieties is operating to spread the means. In such cases the variety having the higher mean will generally be found to yield higher values at all, or almost all stations, and for all practical purposes the varieties must therefore be considered to differ in malting quality.

The main data for the investigation are presented in summarized form, as varietal means over all stations, in the body of Table I. The varieties are listed in each column in descending order with respect to the malt quality in question. To facilitate examination of the data, only the names of those varieties which changed their positions have been listed in the columns for eight-day malts.

TABLE I

MEAN VALUES FOR SIX- AND EIGHT-DAY MALTS

Extract, %				Diastatic power, °L.				Perm. sol. nitrogen as % of wort solids				Malting loss, %			
6-day		8-day		6-day		8-day		6-day		8-day		6-day		8-day	
Mean values over all samples															
	72.8		73.0		142		155		1.16		1.33		7.6		11.1
Necessary differences required for a 5% level of significance															
	1.2		1.3		15		16		0.10		0.11		0.6		0.8
Mean values, over all stations, for each variety															
Olli	75.3		75.4	Olli	187		206	Olli	1.34		1.51	Olli	8.6		12.1
O.A.C. 21	73.5	Rex	74.0	Peatland	158		175	Rex	1.24		1.44	O.A.C. 21	8.0	Rex	11.5
Rex	73.4	O.A.C.21	73.4	Pontiac	151	Rex	161	Peatland	1.21	Velvet	1.39	Pontiac	8.0	O.A.C.21	11.4
Pontiac	72.7		72.8	Rex	145	Pontiac	158	Velvet	1.16	Peatland	1.38	Peatland	7.6	Velvet	11.1
Nobarb	72.4		72.7	O.A.C.21	145	Velvet	154	O.A.C.21	1.14		1.27	Rex	7.4	Pontiac	11.0
Peatland	72.2		72.3	Velvet	138	O.A.C.21	152	Pontiac	1.11		1.27	Velvet	7.3	Peatland	11.0
Regal	71.6		71.7	Nobarb	117		134	Regal	1.07		1.19	Regal	7.3		10.7
Velvet	71.2		71.6	Regal	96		103	Nobarb	0.96		1.15	Nobarb	6.8		10.1

While the data show several changes of varietal position between the six- and eight-day series, in only one instance is the change significant. Thus, with respect to extract, O.A.C. 21 and Rex reversed their positions, but the difference of 0.6% between them cannot be considered significant. Two pairs of varieties, Pontiac and Rex, and O.A.C. 21 and Velvet, reversed their positions with respect to diastatic power, but neither pair can be considered to differ significantly in either series. In permanently soluble nitrogen, Velvet and Peatland changed positions, but the differences between them are only half the required amount. Five varieties were involved in a change of order with respect to malting loss. Of these, Rex and Pontiac changed their relative position most and present the exception mentioned in the first sentence of this paragraph. The malting loss for Pontiac was just significantly higher than that for Rex in the six-day series, whereas in the eight-day series Rex had the higher malting loss. It is apparent that the two-rowed variety Rex did not grow as rapidly as some of the six-rowed varieties during the initial germination period but continued to grow more vigorously during the extra two days. This hypothesis is supported further by the data for extract.

Besides considering complete reversals of positions, it is also necessary to consider changes in the spreads between varieties which do not involve a reversal. These latter changes can be observed more readily by reference to Table II. The data represent differences between the mean increase over all varieties from six- to eight-day malts and the increase for the variety listed in the first column. A positive sign indicates that the value for the variety in question increased more than the average amount, and a negative sign indicates that it increased less. Thus, in any column, the varieties represented by the greatest positive and negative values changed their relative positions most.

TABLE II

DIFFERENCES BETWEEN AVERAGE INCREASE OVER ALL VARIETIES BETWEEN SIX- AND EIGHT-DAY MALTS AND INCREASES FOR INDIVIDUAL VARIETIES

Variety	Extract, %	Diastatic power, °L.	Perm. sol. nitrogen	Malting loss, %
Olli	−0.1	+ 6	0	+0.1
O.A.C. 21	−0.3	− 6	−0.05	0
Rex	+0.4	+ 3	+0.03	+0.6
Pontiac	−0.1	− 6	−0.01	−0.4
Nobarb	+0.1	+ 4	+0.02	−0.2
Peatland	−0.1	+ 4	0	−0.1
Regal	−0.1	− 5	−0.05	−0.1
Velvet	+0.2	+ 3	+0.06	+0.3
Mean increase over all varieties	+0.2	+13	+0.17	+3.5

It turns out that with respect to extract, the varieties which changed their relative position most were those which also reversed their positions, namely, O.A.C. 21 and Rex, and these cannot be considered to differ significantly.

In diastatic power, the difference between Olli and O.A.C. 21, and between Olli and Pontiac, increased by 12° Lintner, an amount which approaches the order of magnitude of the necessary difference based on the over-all differential effect of environment on varieties. With respect to permanently soluble nitrogen, the greatest change in relative positions occurred with Velvet on the one hand and O.A.C. 21 and Regal on the other. The increase in the difference amounts to 0.11, which equals the necessary difference. The data for malting loss show that the greatest change in position also led to a reversal in position. It concerns Rex and Pontiac and was discussed above.

The results of the study again suggest that the differential effect of malting method on varieties is not as large as the differential effect of environment. The former, nevertheless, constitutes an appreciable source of error in the interpretation of the results of routine malting tests. As a result, it is apparent that supplementary investigation of all reasonably promising varieties will be required before an adequate estimate of their malting qualities can be obtained.

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STUDIES ON FOOT- AND ROOT-ROT OF WHEAT

VI. METHODS OF SECURING INFECTION OF WHEAT SEEDLINGS FOR STUDY IN NUTRIENT SOLUTIONS[1]

By W. C. Broadfoot[2] and L. E. Tyner[3]

Abstract

The two foot-rot diseases of wheat caused by *Helminthosporium sativum* P. K. & B. and *Fusarium culmorum* W. G. Sm. were studied in nutrient culture solutions instead of in the usual substrates of soil or sand. The most satisfactory results were obtained by first germinating the grains in a specially designed tray, then securing infection of the young plants by adding inoculum to the tray, after which the seedlings were transplanted to the nutrient culture solution. Infection of the seedlings was distinctly increased when sucrose was added to a nutrient solution infested previous to the time of transplantation. Infection was less satisfactory when the seed was immersed in a spore suspension, dried, and germinated on the tray. Very unsatisfactory infection was secured by adding a spore suspension in water, with or without sugar, to the nutrient solution at the time of transplanting the seedlings. Inoculating the seedlings with a spore suspension by means of a hypodermic needle produced practically no infection. Length of shoot, and particularly the dry and the green weight of the entire plant were reliable quantitative criteria for the evaluation of disease. The first method indicated appears to offer several important advantages in that the degree of infection can be controlled.

It is well known that in the study of the foot-rot disease of cereals, certain very important difficulties in technique have seriously hampered progress. By present methods of artificial inoculation, where concentrated inoculum of the pathogen is placed around or near the grain, the grain itself is often destroyed before it can germinate. If germination occurs, the young seedling may be killed before or soon after emerging from the ground. Should the seedlings survive this ordeal, they are often so severely crippled that a proper study of the natural resistance of the host to the disease becomes practically impossible. On the other hand, if the inoculum is not in direct contact with the grain as it germinates, many plants may escape infection, and thus a disconcerting experimental variable is introduced. If satisfactory infection of the seedlings could be secured by introducing inoculum to the soil at a later period, the problem would be simplified; but on account of the antagonistic action of the soil flora to the pathogen (3, 5), and other associated difficulties of technique, this does not give the desired results.

The purpose of this study was to find a suitable method of producing a uniform but not too severe infection of the seedlings after the grains germinate, so that these difficulties would be avoided.

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[2] *Plant Pathologist, Dominion Laboratory of Plant Pathology, Edmonton, Canada.*

[3] *Plant Disease Investigator.*

Materials and Methods

Infection Technique

Marquis wheat seed was surface disinfected in a 1-1000 mercuric chloride solution for 20 min., washed in running tap water for 60 min., soaked overnight in distilled water at 24° C., and then germinated in special trays. These trays were, with certain slight modifications, essentially the same as used by Hoagland and Broyer (4). The seed was distributed on cheesecloth on a cedar frame, 20 × 40 cm., which was placed in a photographic tray. A similarly covered, but smaller, frame was then placed over the first, about 1 cm. above the seed level. The edges of the cheesecloth on both frames dipped into the water in the bottom of the tray. During germination at 20° C., all trays were covered with glass sheets and brown wrapping paper. Several methods of securing infection were tried. These will be described later in connection with each experiment. Spore suspensions of *Helminthosporium sativum* P. K. & B. and of *Fusarium culmorum* W. G. Sm. were used as pathogens.

Nutrient Solution Technique

The plants were grown in nutrient solutions instead of the usual substrates of soil or sand. Two types of containers were employed, *viz.*, large iron tanks 30 × 30 × 8 in., of 115 litre capacity, coated with asphalt paint, described by Arnon (1), and the ordinary glazed earthenware, one-gallon crocks. The covers of the tanks held 64 corks, each supporting one plant. The sheet metal tops of the crocks, coated with paraffin wax, held five corks, each supporting one plant.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS IN CC. PER LITRE

No.	Nutrient solution	$Ca(NO_3)_2$ M/1	KNO_3 M/1	$MgSO_4$ M/1	KH_2PO_4 M/1	K_2SO_4 M/.5	$Ca(H_2PO_4)_2$ M/.01	$CaCl_2$ M/1
1	Complete	5	5	2	1			
2	Low potassium	6.75	1.5	2			50	
3	High potassium	5	5	2	1	9		
4	Low nitrogen	1.25	1.25	2		8.75	50	
5	High nitrogen	8.75	8.75	2	1			
6	Low calcium	1.25	10	2	1			
7	High calcium	5	5	2				3.75

The nutrient culture solutions were varied according to need from basic formulas which were kindly furnished by Professor D. R. Hoagland of the University of California. The compositions of the nutrient solutions employed in all experiments reported are given in Table I. The nutrients were added to the tanks and to the crocks in amounts proportional to 115 and 4 litres, respectively. Iron, as ferric tartrate, at the rate of 1 cc. of 0.5% solution per litre, was added once a week in Experiments I, II, III and IV, and twice a week in Experiments V and VI. The pH of the nutrient solutions was also determined colorimetrically.

Other Technique

While the experiments were in progress, four 500-watt lights, suspended three feet above the greenhouse bench, were turned on from about 4 to 12 p.m.

each day. The plants were taken up approximately 40 days after they were transplanted. At this time, the length of shoots and roots, and their green weight were recorded. The plants were then dried in an oven at 90° C. for 48 hr., and weighed. Where possible, the experimental data were tested by Fisher's (2) Analysis of Variance method, and by the "F" test of Snedecor (6) to determine the significance of the differences observed among the various treatments. Other necessary details of technique will be supplied in conjunction with each experiment.

Experimental Results

The methods of securing infection which proved unsatisfactory were: *(a)* a spore suspension of the pathogen injected into the crown area of the seedlings by means of a hypodermic needle; *(b)* a spore suspension of the pathogen in water, or *(c)* in sucrose solution, added to the nutrient solution in the tanks at the time of transplanting the seedlings; *(d)* the seed soaked in a spore suspension of the pathogen prior to its germination. The results from the four methods indicated are reported in Experiments I, II, III and IV, respectively.

The methods which gave satisfactory infection were: *(a)* a spore suspension of the pathogen, plus sugar, added to the nutrient solution in the tanks some time prior to transplanting the seedlings; *(b)* the roots of the seedlings immersed in the inoculum prior to transplanting them to the tanks. The results from these two methods are reported in Experiments V and VI, respectively.

Inoculation with Hypodermic Needle (Experiment I)

On October 25, 1935, Marquis wheat grains were spread on trays to germinate. Five days later, 64 uniform seedlings were transplanted to each of the six 115-litre tanks, previously described. Two tanks contained a complete nutrient solution, two others a high-calcium solution, and the remainder a low-calcium solution. The compositions of the nutrient solutions are given in Table I, Nos. 1, 6 and 7. Eleven days later, 1 cc. of a spore suspension of *H. sativum* was injected with a hypodermic needle into the crown tissue of the plants growing in one of each pair of tanks indicated above. Similar amounts of sterile distilled water were injected into the crowns of the plants growing in each of the three corresponding control tanks. This experiment was taken up on December 6. The results are given in Table II.

TABLE II

EFFECT ON DISEASE EXPRESSION* OF A SPORE SUSPENSION OF *Helminthosporium sativum* INJECTED HYPODERMICALLY INTO THE CROWNS OF WHEAT SEEDLINGS

Nutrient solution	pH of solution		Shoot length, cm.		Root length, cm.		Total length, cm.		Dry weight, mg.	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
Complete	7.1	7.1	36.4	37.4	29.9	38.3	66.3	75.7	328.1	364.1
Low calcium	6.5	6.5	43.1	42.6	42.8	45.3	85.9	88.0	437.5	532.8
High calcium	7.1	7.0	41.0	38.1	33.1	38.3	74.0	76.4	400.0	375.0

* *In terms of plant growth.*

These indicate that a very slight amount of disease developed in plants in any of the nutrients. Therefore, this method of securing infection was ineffective. The length of roots and length of the plants in the inoculated series were a little less than in the control series of the corresponding nutrient solution, but there was no difference in length of shoots. However, the dry weight of the plants in the complete solution and in the low-calcium solution was respectively 10 and 18% less than the dry weight of the plants in the corresponding control tanks.

Inoculation by Adding Spore Suspension, without Sugar, to Tank Solution (Experiment II)

This experiment, which was begun December 10, 1935, and harvested January 20, 1936, was carried out in the six 115-litre tanks used in Experiment I. The complete nutrient solution was the same as in that experiment, but instead of low- and high-calcium solutions, low- and high-nitrogen solutions were substituted. The composition of these solutions is indicated in Table I, Nos. 1, 4 and 5.

Marquis wheat seedlings of uniform size were transplanted to all tanks. These seedlings were from seed set to germinate five days before. The inoculum consisted of a heavy spore suspension from colonies of *H. sativum* 30 days old. One tank of each nutrient solution pair received inoculum, the control did not.

The results, which are given in Table III, indicate that the disease did not develop satisfactorily in any of the solutions. On the contrary, the plants in all three inoculated tanks were decidedly greener, and had longer shoots and roots than the plants which grew in the control tanks. The dry weight of the plants grown in the high-nitrogen solution plus pathogen was 10% less than that in its control. In the other two solutions, there was no difference in dry weight of plants in the infested and control series.

TABLE III

EFFECT ON DISEASE EXPRESSION* OF ADDING A SPORE SUSPENSION OF *Helminthosporium sativum*, WITHOUT SUGAR, TO NUTRIENT SOLUTIONS AT TIME OF TRANSPLANTING WHEAT SEEDLINGS

Nutrient solution	pH of solution		Shoot length, cm.		Root length, cm.		Total length, cm.		Dry weight, mg.	
	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Complete	7.1	7.1	30.8	27.9	27.5	26.1	58.3	54.1	173.4	175.0
Low nitrogen	6.5	6.7	49.5	46.6	51.7	42.1	101.2	88.7	437.5	418.8
High nitrogen	7.0	7.1	39.0	35.5	44.6	41.1	83.6	76.6	276.6	309.4

* *In terms of plant growth.*

Inoculation by Adding Spore Suspension with Sugar to Tank Solution (Experiment III)

This experiment, which ran from January 25 to February 14, 1936, was similar in plan to Experiment II, with the exception that the solutions were

high or low in potassium rather than nitrogen, and that all tanks received, in addition to the regular nutrient, equal amounts of sugar. The compositions of the nutrient solutions are given in Table I, Nos. 1, 2 and 3.

Uniform seedlings were transplanted to the six tanks five days from the time the seed was placed on the trays to germinate. At this time the pathogen, in the form of a spore suspension, was added to one series of solutions and sugar was added to all tanks. The sugar concentration of each tank was approximately 0.01%. The results of this test are given in Table IV.

TABLE IV

EFFECT ON DISEASE EXPRESSION* OF ADDING A SPORE SUSPENSION OF *Helminthosporium sativum*, WITH SUGAR, TO NUTRIENT SOLUTIONS AT TIME OF TRANSPLANTING WHEAT SEEDLINGS

Nutrient solution	pH of solution		Shoot length, cm.		Root length, cm.		Total length, cm.		Dry weight, mg.	
	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Complete	6.7	6.7	63.9	53.1	53.6	47.3	117.4	100.4	1539.1	1156.3
Low potassium	6.5	6.5	61.5	59.8	48.1	47.0	109.6	106.8	1738.1	1642.9
High potassium	6.7	6.7	54.9	39.3	34.4	44.6	89.2	83.9	1309.5	444.4

*In terms of plant growth.

As in Experiment II, the development of the disease was very light throughout, and, therefore unsatisfactory. However, the dried plants in the complete, low-potassium, and high-potassium solutions with pathogen, weighed respectively, 33, 6 and 195% more, and were definitely taller than the plants from the control series.

Seed Soaked in Spore Suspension of H. sativum prior to Germination (Experiment IV)

This experiment lasted from October 13 to November 19, 1936. The containers used were one-gallon crocks. The complete nutrient solution as employed in Experiments I, II, and III was used as a growth medium.

Seeds of Marquis wheat were disinfected in 1 : 1000 mercuric chloride solution for 20 min., and rinsed for 30 min. in sterile distilled water. One portion of this seed was soaked for 24 hr. in a heavy spore suspension of *H. sativum* 30 days old, and the control portion was soaked for 24 hr. in sterile distilled water. Each lot was then suspended in a desiccator over calcium chloride crystals, dried for five days, and spread to germinate on trays. Seven days later, and each day thereafter for three days, uniform seedlings from each lot were transplanted to the six replicate crocks, five seedlings in each. The data from this study are given in Table V.

For the inoculated series, the "F" value exceeds the 1% point for length of shoot, and the 5% point for total length of shoot and roots. It is also significant for both green and dry weight of the plants. On the other hand, the development of disease was not very satisfactory, as apparently certain seedlings on the tray escaped infection, some were killed outright, while others

developed only a slight amount of disease. Thus, although distinctly better results were obtained by this method than by the other three methods, the technique obviously does not favor a sufficiently uniform infection to recommend its use when the plants are grown in nutrient solution.

TABLE V

EFFECT ON DISEASE EXPRESSION* OF SOAKING WHEAT SEED IN A SPORE SUSPENSION OF *Helminthosporium sativum* AND TRANSPLANTING THE SEEDLINGS TO COMPLETE NUTRIENT SOLUTION AT VARIOUS INTERVALS

Interval, days	Shoot length, cm.		Root length, cm.		Total length, cm.		Green weight†, gm.		Dry weight†, gm.	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
7	32.9	34.7	30.4	31.9	63.3	66.6	11.6‡	17.3	1.16‡	1.65
8	31.5‡	35.7	31.0	32.6	62.5‡	68.4	10.9‡	15.3	1.06‡	1.47
9	31.1	33.5	32.6	33.1	63.7	66.7	11.1‡	15.0	1.17‡	1.50
10	32.5	31.7	35.1	36.9	67.6	68.6	14.2‡	17.3	1.33‡	1.63
$2 \times \sqrt{2} \times$ S.E.	2.7		4.5		5.4		2.7		.23	
"F" Inoculations	8.35		1.49		5.88		41.21		43.62	
"F" Intervals	1.78		3.66		1.17		2.78		2.53	
"F" Replicates	1.03		2.37		2.70		1.88		.84	

The 5% points for inoculations, intervals, and replicates are 4.08, 2.84, and 2.45, respectively; and the 1% points are 7.31, 4.31, and 3.51, respectively.

* *In terms of plant growth.*

† *Average weight of five plants.*

‡ *Indicates significant values between inoculated and control series.*

Inoculum, plus Sugar, Added to the Nutrient Solution Prior to Transplanting Seedlings (Experiment V)

This experiment lasted from March 19 to April 8, 1936. The six 115-litre tanks used in Experiments I, II and III were employed in this study. A nutrient solution with a high concentration of potassium was used in all tanks, because of the extra vigor which this solution plus the pathogen had given to the plants in Experiment III. Sufficient sugar was added to the solution in one pair of tanks to make the concentration 0.1%, and that of the second pair 0.3%. Sugar was not added to the third pair.

Two days prior to transplanting the seedlings to the tanks, equal amounts of a spore suspension of *H. sativum* were added to one unit of each pair. This suspension was the same as used in all previous experiments, that is, spores from colonies 30 days old, grown in ten large test tubes. The seedlings were transplanted, 64 per tank, seven days after the seed was set to germinate on trays. The results of this study are presented in Table VI.

Fifteen days after transplantation, the plants became distinctly chlorotic in the control tank which received no sugar and no inoculum. After the eighteenth day, ferric tartrate was added twice each week to the solution in all tanks, instead of once each week, as had been done in all previous experiments. Within three days the chlorosis began to disappear. But the point of interest is that at the conclusion of this experiment, in the unit to which the pathogen was added, the lengths of the shoots, of the roots, and of the whole plants were about 10, 14, and 13%, respectively, longer than for

the plants in the corresponding control units. In contrast to these results, in the other two series to which pathogen was added, but with 0.1 and 0.3% of sugar, respectively, the plants were distinctly shorter in length of shoot and of root, and the dry weight was much less than that in the corresponding control series. In other words, the addition of sugar to the nutrient solution promoted infection by the pathogen, and thereby increased the development of the disease.

TABLE VI

EFFECT ON DISEASE EXPRESSION* OF ADDING A SPORE SUSPENSION OF *Helminthosporium sativum*, PLUS VARIOUS CONCENTRATIONS OF SUGAR, TO A HIGH-POTASSIUM SOLUTION, TWO DAYS PRIOR TO TRANSPLANTING WHEAT SEEDLINGS

Sugar, %	*pH of solution*		Shoot length, cm.		Root length, cm.		Total length, cm.		Dry weight, mg.	
	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
0	6.7	6.8	36.1	32.6	44.8	39.2	80.9	71.7	509.4	559.4
0.1	6.6	6.7	26.2	37.4	8.0	19.9	34.2	57.3	192.2	625.0
0.3	6.6	6.7	20.0	25.6	5.9	9.2	25.9	34.8	94.7	165.6

* *In terms of plant growth.*

Inoculation of Roots of Seedlings Prior to Transplanting (Experiment VI)

The duration of this experiment was from October 1, 1936, to November 4, 1936. One-gallon crocks were used. These were filled with the complete nutrient solution, without the addition of other minerals, sugar, or a spore suspension of the pathogen. Infection of the seedlings was secured by immersing their roots for various periods in inoculum of *H. sativum*. This inoculum was prepared by incubating the pathogen for ten days in a complete nutrient solution to which a 2% solution of sugar was added. Five days after the seed was set to germinate on trays, the roots of the seedlings were placed in the inoculum. At the end of seven days, and also each day thereafter for six days, uniformly infected seedlings were transplanted to the six replicate crocks, five seedlings in each. The seedlings for the corresponding controls were germinated on another tray at the same time, and the roots were immersed in the nutrient solution for the same intervals as given the corresponding inoculated units. The seedlings were then transplanted to the control crocks. The results are presented in Table VII.

The "F" value for length of shoot, length of roots, total length of shoot and roots, green weight, and dry weight of the plants indicated very significant differences for the various inoculation periods. In other words, the severity of the disease depended upon the time the roots of the seedlings were immersed in the inoculum. Thus, by suitable manipulation of the stage at which the roots are placed in contact with the pathogen, and the duration of the contact, it appears that the severity of the subsequent development of the disease can be reduced or increased as desired.

The foregoing technique was employed in testing the disease reaction of wheat seedlings to *Fusarium culmorum*, with essentially the same results as have just been reported for *H. sativum*.

TABLE VII

EFFECT ON DISEASE EXPRESSION* OF IMMERSING ROOTS OF WHEAT SEEDLINGS IN INOCULUM OF *Helminthosporium sativum* FOR VARIOUS INTERVALS, THEN TRANSPLANTING TO COMPLETE NUTRIENT SOLUTION

Interval, days	Shoot length, cm.		Root length, cm.		Total length, cm.		Green weight†, gm.		Dry weight†, gm.	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
7	28.3‡	34.0	34.8‡	41.3	63.1‡	75.3	9.9‡	17.6	.89‡	1.64
8	28.7‡	33.8	34.4‡	42.0	63.1‡	75.8	9.6‡	15.9	.84‡	1.46
9	28.9‡	33.1	33.1‡	39.0	62.0‡	72.1	9.1‡	15.3	.83‡	1.37
10	27.3‡	32.2	32.1	36.2	59.4‡	68.3	7.5‡	11.3	.72‡	1.02
11	25.8‡	30.3	28.0‡	34.7	53.7‡	65.3	5.7‡	11.7	.55‡	.99
12	23.5‡	30.4	25.6‡	31.2	49.0‡	61.6	5.9‡	12.0	.49‡	1.12
13	24.7‡	30.9	24.6‡	33.7	49.3‡	64.6	6.3‡	13.2	.50‡	1.14
$2 \times \sqrt{2} \times$ S.E.	2.4		4.1		5.9		1.7		.15	
"F" Inoculations	111.13		52.35		85.33		285.66		274.81	
"F" Intervals	7.11		11.69		11.82		18.30		19.99	
"F" Replicates	2.07		.98		.55		.36		.65	

The 5% points for inoculations, intervals, and replicates are 3.98, 2.23, and 2.35, respectively; and the 1% points are 7.01, 3.07, and 3.29, respectively.

* *In terms of plant growth.*

† *Average weight of five plants.*

‡ *Indicates significant values between inoculated and control series.*

Discussion

These studies have indicated that a satisfactory method of securing infection of wheat seedlings with *H. sativum* or *F. culmorum* is to germinate the seedlings, and then, before they are transplanted, to immerse their roots for about seven to ten days in a nutrient solution in which the pathogen has been cultured. By delaying contact with the inoculum, the roots get a fair opportunity to develop; by decreasing or increasing the duration of the contact, the severity of the attack in the seedling stage may be more or less controlled at will. This technique permits good development of disease in a nutrient solution, and the method appears to possess advantages, should the plants be transplanted to a soil or sand substrate instead of to a nutrient solution. However, this possibility has not yet been demonstrated by experiment. Another advantage is that one may choose uniformly infected seedlings for study, which is important. (Experiment VI.)

Some infection and fairly satisfactory subsequent development of the disease were secured by adding a spore suspension of the pathogen and sugar directly to the nutrient solution, a few days prior to transplanting the seedlings. However, this method does not permit of the same degree of control or as much accuracy as is possible with the previous method. (Experiment V.)

On the other hand, when the spore suspension was added at the same time that the seedlings were transplanted, practically no disease developed. The addition of sugar to the solution made no appreciable difference in the results obtained. (Experiments II and III.)

Rather poor and uneven infection was secured when the seed was soaked in a spore suspension of *H. sativum* prior to germinating the seed. There

appears little to recommend this method in connection with the study of the disease in nutrient solutions. (Experiment IV.)

Of all the methods studied, the injection of spores of the pathogen into the crown tissue of the seedlings was least successful in producing infection. (Experiment I.)

Although a study of the relation of the composition of the nutrient solution to the development of the disease was not the primary purpose of the present paper, the evidence obtained from the above experiments indicated that this angle of the problem should be studied in detail. This is being done and the results will be reported later. Suffice it to say now that the plants were larger and more vigorous in certain nutrient solutions than they were in others. For example, in Experiments I, II and III, where no disease developed, the plants were less vigorous in the solutions having a high concentration of calcium, nitrogen, or potassium than they were in those with a low concentration of these minerals. With one exception, namely, the solution with a high potassium concentration (Experiment III), the plants were less vigorous in the complete nutrient solution. Apparently satisfactory development of disease occurred in the complete nutrient solution. (Experiment VI.)

A point of particular interest was that, in those experiments where very little or no disease developed, growth was better and chlorosis was less in certain solutions to which the pathogen was added as a spore suspension than in the controls. This occurred in all infested solutions of Experiments II and III. Evidently normal chlorophyll production in the plants was maintained in the presence of *H. sativum* or of *F. culmorum*, but not in the ahsence of either pathogen, despite the fact that ferric tartrate was added once per week. A tentative explanation is that these pathogens either increase the availability of iron in the solution or favor its assimilation by the plant. The importance of further study of this phenomenon, and its relation to the development of disease in culture solutions, is emphasized. The results of the experiments on this phase, now in progress, will be reported later.

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FRACTIONATION OF NITROGEN IN DEVELOPING WHEAT KERNELS[1]

BY A. G. MCCALLA[2]

Abstract

Total nitrogen in developing wheat kernels was fractionated to give non-protein nitrogen and three protein fractions. Each fraction was hydrolyzed and analyzed for amide and arginine nitrogen.

All fractions increased in amide and decreased in arginine nitrogen during kernel development. The main portion of the water-soluble protein is static in nature, and is considered to play no part in the metabolism of the endosperm proteins. The trend, with time, of amide in the two other protein fractions (soluble and insoluble in normal potassium iodide) was closely parallel to the trend of amide in non-protein nitrogen. The chemical nature of each of the gluten fractions in flour from the most insoluble to the most soluble is determined by the chemical nature of successive portions of the non-protein nitrogen in the wheat kernel at progressive stages of maturity.

Introduction

A considerable amount is known concerning the course of moisture, total nitrogen, and gross nitrogen fraction changes which take place during the development of grain, but little study has been made of the nature of the gross protein fractions, nor of the way in which the more complex proteins of the endosperm are formed.

Woodman and Engledow (9), Knowles and Watkin (3), and Teller (8) have all separated the total nitrogen of developing wheat kernels into non-protein and various protein fractions. Knowles and Watkin and Teller, however, dried their material before analyzing it, so the data obtained are not very useful in appraising metabolic changes. This criticism applies to other studies which have been carried out with wheat, and also to many made with other grains. Woodman and Engledow, however, worked with fresh material. They partitioned the non-protein nitrogen into various types of nitrogen compounds, but made only a gross separation of the protein fractions. No detailed analysis of these fractions was made.

Investigations carried on in this laboratory during the past few years have been concerned on the one hand with the total nitrogen and nitrogen fractions of developing wheat (4, 5), and on the other with fractionation of the protein

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[2] *Research Assistant, Associate Committee on Grain Research, University of Alberta, Edmonton.*

making up the gluten of flour (6). The present study combines the basic methods of both lines of investigation, with the object of obtaining information as to the way and order in which the various nitrogen fractions are formed in the developing kernel.

Material and Methods

The experiments were carried out on material grown at Edmonton in the summer of 1935. Two varieties of hard red spring wheat, Reward and Red Bobs, were grown side by side in one-tenth acre plots. When the lower florets were in full flower, 3,500 heads of each variety at the same stage of development were tagged. There was a difference of two days in the time of tagging the two varieties. On July 26, eight and ten days after tagging, the first collection was made. The heads were taken to the laboratory where approximately 300 of each variety were threshed out. The fresh kernels were used as soon as possible for the various determinations. Collections were made twice a week until development of the grain was complete. Fewer heads were collected at later dates, the number falling to approximately 100 at maturity.

The general plan of analysis is presented in outline on the opposite page.

Grinding of the fresh kernels was relatively easy to accomplish until the dry matter content reached 45 to 50%, but became increasingly difficult with maturity. All collections of Red Bobs were satisfactorily ground and extracted, but a few of the more mature Reward samples yielded too high values for the KI-insoluble nitrogen. The difference was due to the harder, more vitreous texture of the Reward kernels, which made disintegration much more difficult.

Normal potassium iodide was used as an extraction solution for several reasons. Many other substances have been used for extracting wheat proteins, but only two seemed to warrant serious consideration. These were sodium salicylate solution, which is an excellent dispersing agent for gluten, and a combination of dilute salt solutions followed by alcohol. The use of sodium salicylate presented technical difficulties which were almost impossible to overcome. Complete precipitation of the protein dispersed cannot be secured with salts, and acid precipitants bring down salicylic acid. Furthermore, this reagent disperses some of the starch to a jelly-like mass which cannot be centrifuged or filtered off. Salts other than potassium iodide dispersed too little of the protein remaining after water extraction. When dilute salt solution was followed by 70% alcohol, the resultant extract was much more difficult to handle than that obtained with normal potassium iodide. The latter dispersed approximately 50% of the protein remaining after water extraction of the ground wheat, and appeared to be the most generally satisfactory reagent to use.

After precipitation of the KI-soluble protein, small amounts of nitrogen remained in the filtrate. These were determined and are included as KI-soluble. At no time after the kernels were well formed did this nitrogen make up more than 5% of the fraction.

Scheme of Analysis

Freshly threshed kernels

1. Large samples for green and dry weight per 1000 kernels.
2. Aliquot samples: for dry matter determination, dried 48 hr. *in vacuo* at 98° C.; and for total nitrogen determination by the Kjeldahl method.
3. Aliquot samples for nitrogen fractionation: 20 to 40 gm. (approximately 300 mg. N) ground repeatedly in a mortar, transferred to centrifuge bottle and extracted with distilled water for 1 hr. with continual shaking. Centrifuged and re-extracted twice.

- Supernatant liquid transferred to Erlenmeyer flask and 50% CCl_3COOH added to make a 2·5% solution.
  - Filtered
    - Filtrate containing non-protein N hydrolyzed in 5% H_2SO_4 for 2 hr. Hydrolyzate filtered hot and thoroughly washed with hot water.
      - Solids (Humin N)
      - Filtrate
        1. Aliquot for total N.
        2. Aliquot nearly neutralized with concentrated NaOH. Neutralized with MgO milk and aerated 1 hr.
           - Ammonia (Amide N)
           - Residue made up to 20% NaOH. Refluxed 6 hr.
             - Distilled
               - Ammonia (Arginine N × ½)
               - Residue (Discard)
    - Solids containing water-soluble protein hydrolyzed in 20% HCl for 24 hr. Subsequent treatment same as for non-protein N.
- Solids extracted with N KI solution for 1 hr. with continual shaking. Centrifuged and re-extracted twice.
  - Supernatant liquid transferred to Erlenmeyer, and 50% CCl_3COOH added to make a 2.5% solution.
    - Filtered
      - Filtrate all used in total N determination.
      - Solids containing KI-soluble protein treated the same as those containing water-soluble protein.
  - Solids containing insoluble protein treated the same as those containing water-soluble protein.

The hydrolysis of the KI-insoluble fraction in the presence of large quantities of carbohydrates results in the formation of relatively high amounts of humin. While this humin formation may reduce the arginine values, it has been shown that the ammonia set free by hydrolysis of the amide groups is not affected (2). The amide values, therefore, are probably quite reliable.

Other details or references for individual methods of determination are given in an earlier paper (4).

Results

The results of dry matter and total nitrogen determinations are not presented since they add nothing to the results and conclusions of earlier studies (5, 9). Percentage dry matter is used throughout this paper as a measure of maturity, since the critical changes in metabolism have been found to be associated with definite levels of dry matter content.

In the discussion, all results for any single determination are treated as a unit, regardless of the variety of wheat. In so far as the main conclusions are concerned, the two varieties behaved alike. Any differences which were found are associated with the differences in general level of protein content, Reward being at all stages appreciably higher than Red Bobs. Since Reward was more difficult to grind than Red Bobs, the values for the quantities of the various fractions extracted are not as reliable for this variety.

Nitrogen Fractions

The results of the nitrogen fractionation are presented in Table I and Fig. 1.

TABLE I

Nitrogen fractions as % of total nitrogen in developing wheat

Dry matter content, %		Non-protein nitrogen, %		Water-soluble protein nitrogen, %		KI-soluble protein nitrogen, %		KI-insoluble protein nitrogen, %	
Reward	Red Bobs	Reward	Red Bobs	Reward	Red Bobs	Reward	Red Bobs	Reward	Red Bobs
25.8	25.2	31.1	37.5	36.4	31.5	—	—	—	—
28.8	26.8	27.6	35.6	34.4	35.7	17.7	12.8	19.6	16.0
32.5	29.9	22.8	29.7	31.4	36.4	20.8	16.1	26.4	21.4
38.0	35.0	—	—	27.4	33.8	24.8	22.6	32.8	26.0
41.4	39.3	14.8	18.0	20.9	28.3	29.0	26.2	37.5	26.0
44.7	43.2	14.6	16.9	15.2	22.5	28.3	28.3	38.9	31.8
47.0	46.9	11.9	13.1	13.8	15.6	27.3	31.1	46.2	40.6
50.6	48.8	9.4	9.8	14.3	15.5	32.3	33.7	44.3	44.2
53.8	52.5	7.8	8.9	11.6	13.0	35.1	36.7	44.6	38.6
56.0	56.1	6.5	7.3	10.7	10.2	32.8	41.0	48.2	41.0
62.4	58.8	7.8	6.8	10.1	7.6	40.1	42.2	40.0	43.3

Both the precentages and total weights of non-protein and water-soluble protein nitrogen agree with values presented by Woodman and Engledow (9) and McCalla and Newton (5). The water-soluble protein is, of course, extracted with a dilute salt solution, since the grain contains appreciable amounts of salts.

The protein soluble in normal potassium iodide formed slightly less than half that remaining after extraction with water. The amount of this fraction increased most rapidly after the wheat reached a dry matter content of about 45%.

The protein insoluble in normal potassium iodide is the least definite fraction of all those studied. It consists of that part of the endosperm protein

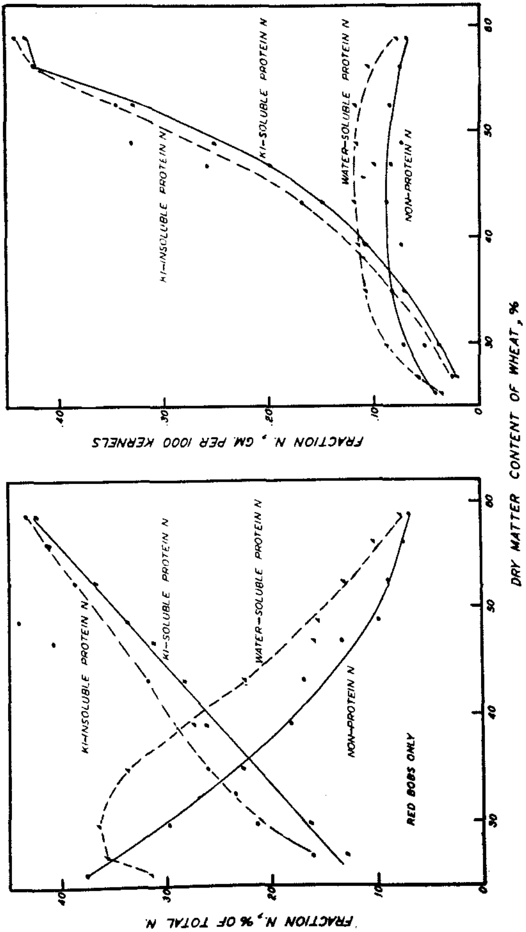

FIG. 1. The distribution of nitrogen fractions in developing kernels of Red Bobs wheat.

which is insoluble in this reagent, plus the relatively insoluble proteins of the bran layers and structural units, plus any amount of the other fractions remaining as a result of incomplete extraction. Nevertheless, the general course of the results shows that there was a fairly regular increase in this fraction with maturity, and the increase was certainly largely the result of an increase in the more insoluble fractions of the gluten protein. The two points (Fig. 1) which fall off the general curve were obtained with inadequately ground wheat. When precautions were taken to insure reasonably complete extraction of the more soluble fractions, no such discrepancies occurred. The part of this fraction contained in the bran and other structural material is largely developed at an early stage, and if allowance is made for a constant quantity throughout development of the grain, the values for, but not the course of, the results would be slightly altered.

The curves in the second part of Fig. 1 are very similar to those presented by Bishop and Marx (1) for the proteins of barley.

Analyses of Nitrogen Fractions

Each of the fractions was hydrolyzed and analyzed for amide and arginine nitrogen. The results are presented in Fig. 2.

The accuracy with which amide nitrogen can be determined makes all the values reliable. The experimental error of the arginine determination is, however, much greater, with the result that the values obtained for the non-protein nitrogen fraction are meaningless. The results for the other fractions are much better, but are not as reliable as those for amide. For this reason the general discussion centres around the amide results, although definite trends in arginine exist for each protein fraction. These results, while inadequate in themselves, support the conclusions reached on the basis of the amide data.

There were marked differences in the amide values obtained for the four fractions, and in the changes in the proportion of amide during maturation of the grain. There was, however, a very definite and regular change with each fraction.

Osborne (7) concluded that the albumin and globulin of wheat are contained chiefly in the embryo, while the gluten proteins make up most of the protein material found in the endosperm. Woodman and Engledow (9) extended this conclusion, as a result of finding that the gluten proteins accumulated continuously with maturity, but the embryo proteins, to which the NaCl-soluble protein of their study chiefly belonged, did not. They raised the question as to whether these embryo proteins functioned as precursors of the gluten proteins, but did not speculate on this point.

The results of the present study seem to offer at least a tentative answer to this question. Since the amide content of the water-soluble protein changed less with maturity than that of any other fraction, and since the level of amide content was at all stages of maturity much lower than that of either the non-protein nitrogen or the more insoluble protein fractions, it would appear

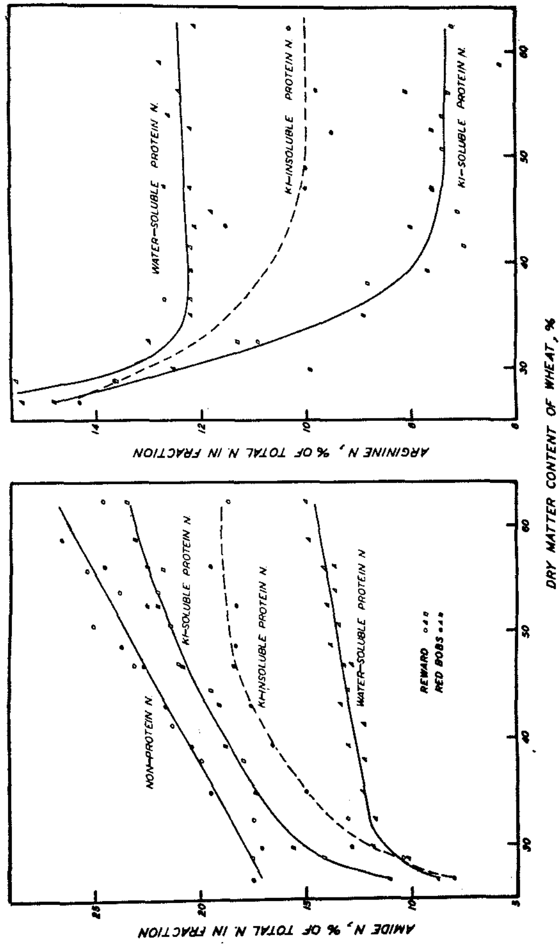

FIG. 2. Amide and arginine nitrogen in the various nitrogen fractions of developing wheat.

that at least the main portion of this water-soluble protein is not active in metabolism. If this view is correct, then production of the endosperm proteins must take place directly through the condensation of the compounds making up the non-protein nitrogen.

While the main portion of the water-soluble protein is thus considered to be static in nature, there must be a small portion of this fraction involved in metabolism, since condensation of the soluble non-protein compounds to the relatively insoluble endosperm proteins must take place through a protein fraction intermediate in solubility. This small labile portion of the water-soluble protein should show the same gradual increase in amide nitrogen as exhibited by the other fractions, with progressive maturity. Since this protein is an intermediate step in the metabolism of the main endosperm proteins, the amide content would be intermediate between that of the non-protein nitrogen and that of the KI-soluble fraction; and the presence of small quantities of this protein should cause an increase in amide content of

TABLE II
MIDPOINTS OF FRACTIONS FOR FIG. 3

Dry matter at time of collection, %	Non-protein N*			Gluten N, Flour 2**			
	Mg. N per 1000 kernels	Total mg. N per 1000 kernels	Midpoint of fraction, %	Fraction	Per cent of total dispersed N	Per cent of dispersed gluten N†	Midpoint of fraction, %
25.6	45	45	2.8	1	1.6	1.8	0.9
27.8	60	105	9.4	2	23.4	26.7	15.2
31.2	71	176	18.1	3	15.1	17.2	37.1
36.5	58	234	25.7	4	16.6	18.9	55.1
40.4	73	307	34.0	5	10.1	11.5	70.4
44.0	90	397	44.2	6	13.3	15.2	83.7
47.0	87	484	55.2	7	7.6	8.7	95.7
49.7	80	564	65.7				
53.2	80	644	75.8				
56.0	73	717	85.4				
60.6	80	797	95.0				

* *Mean values for Reward and Red Bobs.*

** *Original data obtained by McCalla and Rose (6), results for only one replicate presented here.*

† *Recalculated from the data in the preceding column, with the assumption that soluble N fractions with low amide values do not belong to the gluten complex.*

the water-soluble fraction with progressive maturity. This increase occurs (Fig. 2), and its magnitude supports the hypothesis that the water-soluble protein is made up of a main static portion which does not change with maturity and a small labile portion which is active in metabolism. Since the range of solubility of these two portions is the same, it is doubtful if the hypothesis can be directly proved.

The amide values for the non-protein nitrogen, on the other hand, indicate a close parallelism between the changes in this fraction and those in the KI-soluble and KI-insoluble protein fractions. The transformation of the non-

protein nitrogen is believed to take place directly to the most soluble fraction of the gluten protein (the small labile portion of the water-soluble fraction), which in turn becomes less soluble. As the kernel fills, there is a continual condensation of non-protein nitrogen to form the endosperm proteins, and a progressive loss in solubility of the protein already formed.

The amide values for non-protein nitrogen have been replotted in Fig. 3 against the midpoints of the non-protein fraction of the successive collections (see Table II). The total quantity of this fraction, assuming that different

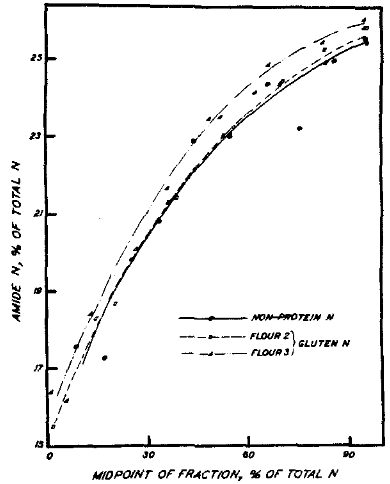

FIG. 3. *Comparison of the amide content of the non-protein nitrogen fraction of wheat at progressive stages of maturity with the amide content of successive gluten fractions of mature wheat.*

portions of the total were determined at each collection, was 797 mg. per 1000 kernels (average for the two varieties). This accounted for 89% of the total KI-soluble and -insoluble nitrogen, all of which must have passed through the non-protein nitrogen stage. The amount not determined was undoubtedly distributed among the various collections, and hence would affect the total quantity but not the trend of the cumulative curve.

With the values for the non-protein nitrogen in Fig. 3 have been plotted the amide values for gluten nitrogen fractions as determined by McCalla

and Rose (6). The amounts of nitrogen in each fraction and the midpoints used in Fig. 3 are given in Table II.*

The agreement between the amide curves for the non-protein nitrogen in developing kernels and for the protein fractions of gluten is remarkably close. It seems probable that the amide content of the least soluble gluten protein is relatively low because this fraction is derived from the non-protein nitrogen present in the kernel at an early stage in the filling process, while the amide content of the most soluble gluten is high because this fraction is derived from the non-protein nitrogen present just before maturity. Similarly, the chemical nature of the intermediate gluten fractions is determined by the chemical nature of corresponding non-protein nitrogen at intermediate stages of maturity.

On the basis of these results the general process of endosperm protein formation may be represented as follows: when filling of the kernel starts, the non-protein nitrogen translocated from the vegetative parts is condensed through a labile water-soluble protein stage to the true endosperm or gluten proteins. As the kernel fills, non-protein nitrogen is continually condensed to form endosperm protein, and the protein already formed gradually loses solubility. Two general effects result from this condensation and decrease in solubility. First, the earliest-formed gluten fractions become the most insoluble portion of the protein in the mature kernel, while the solubility of the later-formed fractions is dependent upon the stage at which these are formed, the last formed being the most soluble. Second, the non-protein nitrogen in the kernel at any one stage of development is the precursor of a definite fraction of the endosperm protein, rather than of a portion of whole gluten complex.

If this hypothesis is correct, the various fractions which make up the gluten of mature wheat would not all be present until the grain is almost fully developed. Chemical development of kernels is practically complete when the dry matter content reaches 58%, and Woodman and Engledow (9) obtained the first tenacious gluten mat at a dry matter content of 55%, and McCalla and Newton (5) obtained it at 56%. The results of the present study agree. Woodman and Engledow concluded that a definite amount of glutenin was necessary before gluten could be formed, since the amount of this most insoluble fraction was low in those collections from which no gluten mat could be obtained. Fractionation studies with gluten show that the presence of the more insoluble fractions is necessary for the formation of a tenacious mass. The results of the present experiment therefore indicate that these more insoluble fractions do not reach the physical state found in the mature kernel until the grain is almost fully developed, although the chemical composition of the fractions is apparently determined at the time that the non-protein nitrogen is condensed to protein.

* *In the work from which these data are taken, it was found that approximately 88% of the gluten nitrogen belonged to a fractionable complex which increased systematically in amide content with increasing solubility. The remainder of the protein was quite distinct in composition, and obviously did not belong to the main gluten complex. The data used in Fig. 3 were recalculated on the assumption that 88% of the dispersed nitrogen makes up the true gluten protein, while the remainder is of the same nature as the static water-soluble protein of the present study.*

One further fact which agrees with the results presented in this paper was recorded in earlier work (4). During the filling of the grain there is a rapid hydrolysis of the protein of the vegetative parts of the plant. The non-protein nitrogen formed by this hydrolysis increased in amide content with time. Since this non-protein nitrogen was translocated to the kernels as fast as it was formed, successive portions of this translocated material were progressively higher in amide. This is reflected in the present study by the increase, with time, of the amide content of the non-protein nitrogen fraction of the kernels.

Further fractionation studies of the nitrogen of developing wheat kernels are planned. Separation of the gluten protein into more numerous fractions should yield more direct evidence as to the validity of the general conclusions presented in this paper.

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CONIOSPORIUM DISEASE OF APPLES AND CRAB-APPLES[1]

By John Dearness[2] and W. R. Foster[3]

Abstract

A new species of Coniosporium—*C. Mali*—parasitic on the crab-apple and several varieties of the common apple, is described. The symptoms of the disease, both on the foliage and the fruit, are similar to those produced by *Fusicladium dendriticum*. Therefore, *Coniosporium scab* is suggested as the common name.

Introduction

In the spring of 1937, Mr. W. H. Robertson, Provincial Horticulturist for British Columbia, directed the attention of the junior writer to what appeared to be a new disease on crab-apple leaves. A survey of the most important fruit districts on Vancouver Island showed that the disease was general not only on crab-apples but also on many varieties of the common apple. One crab-apple tree observed had lost over 75% of its leaves. Defoliation was less severe in apples, hardly reaching 10% in any tree. Owing to the simultaneous presence of Fusicladium scab on the fruit, it was difficult to estimate the damage caused by the newly-described disease, for which the name Coniosporium scab is suggested.

Description of the Fungus

Coniosporium Mali Dearness & Foster sp. nov.—Parasitic on leaves, petioles and fruit of the apple, *Pyrus Malus* L. (*Malus Malus* (L.) Britton).

Discolorations of the green leaf tissue begin as small grayish-brown spots on the veins and veinlets and extending along them, usually branching more or less from them in a dendriform manner (Fig. 2), and becoming almost black with the development of the layer of spores, sometimes darkening the whole upper surface of the leaf (Fig. 1); usually indistinct on the lower surface. On the fruit (Fig. 3), the small scattered acervuli become confluent under the grayish cuticle which is broken into scales and thrown off, producing a scab over the shrunken and cracked pulp, very similar to the scab of *Fusicladium dendriticum* (Wallr.) Fckl. (*Venturia inaequalis* (Cke.) Wint. in its mature stage).

The conidia are olivaceous, seeming sessile or on short, 3 to 13 (20) $\times$ 3 μ, fertile, brown hyphae, rounded or truncate above, tapering downward, often somewhat narrowed near the middle so as to appear slipper-shaped, usually guttate, quite various in shape and size, 13 to 20 $\times$ 3.3 to 8 μ, average about 14.9 $\times$ 5 μ (Fig. 4).

Coniosporium Piri Oudemans on the pear, *Pyrus communis*, is said (1, 3) to have spores 16 μ wide and to be nearly globose. The shape and size of the

[1] *Manuscript received November 25, 1937.*
Contribution from the Plant Pathology Branch, Provincial Department of Agriculture, Saanichton, British Columbia.
[2] *Mycologist, London, Ontario.*
[3] *Assistant Plant Pathologist, British Columbia Department of Agriculture.*

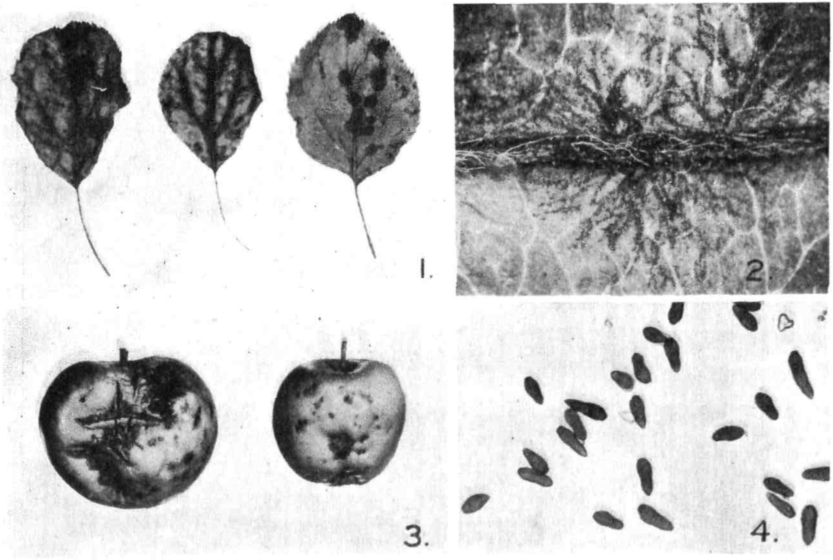

FIG. 1–4. *Coniosporium Mali.* 1. *On apple leaves; somewhat reduced.* 2. *On midvein and veinlets;* 8×. 3. *On fruit; somewhat reduced.* 4. *Conidia; nearly* 400×.

spores of *Coniosporium Rosae* Brun. (2) are not markedly different; it inhabits, however, dead stems of rose and in the description is not said to be parasitic. (*D. No. 8894.*)

Parasiticum; *Fusicladium dendriticum* simulans.

Hab. in foliis viventibus, petiolis et fructu *Piri Mali* L.

Maculis in foliis primitus griseo-brunneis, deinde paene atris, plerumque superioribus, denique confluentibus, interdum tota folia infuscantibus.

Conidiis olivaceis, constipantibus, sessilibus vel in verticibus hypharum fertilium, brevium, fuscarum, oblongis vel sandaleis, diversis in forma et mensura, 13 ad 20 $\times$ 3.3 ad 8 μ, plus minusve 14.9 $\times$ 5 μ. Hyphae fertiles 3 ad 13 (20) $\times$ 3 μ.

Hosts and Distribution

The effects of *Coniosporium Mali* were observed in nearly every apple orchard examined in Sooke, Victoria, Sidney, Duncan, Ladysmith, and Nanaimo districts on Vancouver Island and at one place at Armstrong in the northern Okanagan valley. It was noticeably injurious on crab-apples, on the orchard varieties—Grimes Golden, McIntosh Red, and Vanderpool Red, and present but less injurious on Alexander, Bismarck, Duchess, King, Oldenburg, Salome, Wealthy, and Yellow Newton.

Control

An attempt will be made to complete the life history. The part known, however, is so much like that of the common Fusicladium scab that in all probability the same treatment can be advised for both.

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PHYSICAL PROPERTIES OF MINERAL SOILS OF QUEBEC[1]

PART I. CULTIVATED SOILS

By W. Rowles[2]

Abstract

The relation between texture of soils of Missisquoi and Brome Counties and the growth of alfalfa thereon is examined. It is shown that soils on which alfalfa thrives contain relatively more coarser soil particles. The effect of treatment with lime upon the rate of evaporation of water from the soil, moisture equivalent, heat of wetting, volume weight under field conditions, volume weight of laboratory samples, pore space, moisture content of saturated soil, volume expansion upon wetting, and resistance of the soil to penetration is investigated. Treatment with lime produces a significant effect upon several of these properties. New apparatus is described to measure the volume weight of soil *in situ* and to measure the resistance of the soil to penetration.

Introduction

Although a considerable amount has been written concerning the chemical and biological condition of Quebec soils (11–15), very little has been published of their physical properties. During the past few years a number of studies have been made at this laboratory of the more important physical properties of a group of mineral soils of this province. Most of the work has been done as part of a co-operative study carried on by the Macdonald College Soil Fertility Committee in which the Departments of Agronomy, Bacteriology, Chemistry, and Plant Pathology have also collaborated. As may be noted in the following account, the direction of the physical approach has been influenced, to some extent, by the results obtained by the other departments. The earlier studies were made prior to the acceptance of the present method of mechanical analysis and were concerned entirely with cultivated soils; the later work, described in Part II, was confined to virgin soil.

Experimental Methods and Results

A. Mechanical Analyses

One of the first problems attacked was the inability to grow alfalfa on certain soils in the counties of Missisquoi and Brome. A number of fields were chosen in some of which alfalfa was successfully grown, while in the others all attempts had resulted in partial or total failure. The former were classed as "good" soils, the latter as "poor". Soil samples were taken from these fields as well as from a number of other representative fields of this district, and along with other observations, mechanical analyses were made of both surface and subsoil samples by the pipette method (1). The results are included in Table I.

[1] *Manuscript received May 2, 1938.*
Contribution from the Department of Physics, Faculty of Agriculture of McGill University, Macdonald College, Que., Canada. Journal Series No. 98.
[2] *Associate Professor of Physics, Macdonald College.*

TABLE I

MECHANICAL ANALYSIS OF SOILS FROM MISSISQUOI AND BROME COUNTIES

The separates are made on the American system of particle sizes and the percentages are expressed on the basis of ignited soil. Samples No. 1–6 and 13 are good alfalfa soils; 7-12 and 14 are soils on which alfalfa grows poorly or not at all. Surface and subsoils are indicated by the letters *a* and *b* respectively.

Sample	Stones	Fine gravel	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt	Clay	Loss on solution	Loss on ignition	Total excluding stones
1a	21.4	5.9	7.9	23.4	11.1	4.3	14.4	19.7	1.5	11.3	99.5
1b	44.3	7.4	11.7	27.6	13.4	6.5	11.9	14.5	1.3	7.2	101.5
2a	6.0	3.8	2.3	5.9	9.0	11.1	21.8	25.0	2.5	17.5	98.9
2b	12.8	11.6	2.5	7.3	13.2	17.2	26.1	17.8	0.7	3.2	99.6
3a	15.8	6.5	7.9	22.2	9.3	3.1	18.1	21.7	1.3	10.6	100.7
3b	44.4	10.3	11.8	30.6	11.7	5.8	12.5	11.8	0.6	5.8	100.7
4a	6.2	18.3	3.8	8.8	10.1	10.5	20.5	18.4	1.1	7.7	99.2
4b	6.6	9.1	1.5	4.3	9.6	12.2	26.6	29.5	1.1	5.4	99.3
5a	30.2	18.2	12.1	8.1	4.3	7.0	17.5	15.6	2.1	14.9	99.8
5b	49.8	17.6	22.3	13.3	5.9	7.8	16.4	8.8	1.3	7.5	100.9
6a	19.0	16.1	11.4	20.0	10.4	6.7	16.5	7.6	1.5	9.0	99.2
6b	28.2	14.5	14.8	26.5	14.2	5.3	12.2	4.8	1.3	5.6	99.2
7a	12.7	7.6	2.2	4.8	7.1	11.4	38.3	14.0	2.1	12.7	100.2
7b	22.2	10.3	2.0	4.7	6.9	12.4	24.6	32.2	1.5	5.5	100.1
8a	1.3	0.4	0.6	2.4	17.3	30.5	27.5	4.8	2.4	13.7	99.6
8b	1.3	0.8	0.3	1.8	18.4	39.3	30.6	2.9	1.6	4.2	99.9
9a	4.2	1.1	0.4	1.0	6.7	23.8	40.4	10.1	2.9	13.0	99.4
9b	2.7	0.6	0.3	1.0	10.6	29.6	42.2	9.7	1.8	4.1	99.9
10a	16.2	3.2	1.7	3.6	7.0	17.9	37.8	11.2	2.8	15.5	100.7
10b	14.4	2.7	2.7	5.5	10.5	19.4	44.2	7.2	1.1	7.5	100.8
11a	35.6	6.5	5.7	12.8	16.0	14.3	22.6	11.5	1.8	10.7	101.9
11b	40.5	8.3	8.8	17.0	19.6	15.6	16.2	7.6	1.3	5.7	100.1
12a	23.8	4.1	4.5	6.9	11.7	15.0	27.6	14.4	2.8	12.9	99.9
12b	34.2	3.5	3.4	7.2	12.8	23.6	30.7	12.1	1.5	6.0	100.8
13a	34.0	7.6	7.1	10.9	12.4	8.5	23.4	10.7	3.3	16.1	100.0
13b	51.3	7.5	7.5	11.4	12.6	9.8	23.4	8.8	4.1	14.6	99.7
14a	54.6	4.7	5.5	10.8	9.6	9.7	27.8	15.4	1.6	14.0	99.1
14b	64.8	4.9	6.5	13.3	12.7	13.1	26.2	12.8	1.7	10.0	101.2
15a	9.0	5.7	5.7	15.1	18.6	18.9	18.2	6.5	2.4	8.9	100.0
15b	20.1	4.8	7.6	15.9	21.8	23.0	14.8	4.5	1.9	5.8	100.1
16a	2.2	2.8	4.3	18.4	29.3	14.8	13.1	7.1	1.7	8.4	99.9
16b	1.6	3.2	3.8	19.6	27.7	22.1	14.1	3.4	1.5	4.5	99.9

To compare the relative texture of the good and poor alfalfa soils Table II as been prepared; this shows, in the appropriate columns, the differences between the amounts of the various separates in the good and poor soils, together with their standard errors. By reference to Fisher's t table (5) the differences have been tested for significance. It is seen that the good soils contain a larger percentage of fine gravel, coarse and medium sand, and much less very fine sand and silt in the surface layers. In the subsoil a difference is again evident, there being significantly more fine gravel and less very fine sand and silt in the soils on which alfalfa thrives. While this is an important factor in the problem, there are, no doubt, other contributing causes which should be considered.

TABLE II

COMPARISON OF TEXTURE OF GOOD AND POOR SOILS

Significant differences ($P = 0.05$, $t > 2.45$) are marked *; highly significant differences ($P = 0.01$, $t > 3.71$) are marked **.

—	Surface soils				Subsoils			
	Good soil (mean of 7)	Poor soil (mean of 7)	Difference between means	t	Good soil (mean of 7)	Poor soil (mean of 7)	Difference between means	t
Stones	18.90	21.20	2.30 ± 8.2	0.36	33.91	25.73	8.18 ± 11.0	0.74
Fine gravel	10.91	3.94	6.97 ± 2.60	2.68*	11.14	4.43	6.71 ± 1.99	3.37*
Coarse sand	7.64	2.94	4.70 ± 1.60	2.94*	10.30	3.43	6.87 ± 2.99	2.30
Medium sand	14.19	6.04	8.15 ± 3.31	2.46*	17.29	7.21	10.08 ± 4.62	2.18
Fine sand	9.51	10.77	1.26 ± 1.92	0.65	11.40	13.10	1.70 ± 2.09	0.81
Very fine sand	7.31	17.51	10.20 ± 3.00	3.40*	9.23	21.86	12.63 ± 4.04	3.13*
Silt	18.90	31.71	12.81 ± 2.88	4.55**	18.44	30.67	12.23 ± 4.51	2.71*
Clay	16.96	11.63	5.33 ± 2.69	1.98	13.71	12.07	1.64 ± 4.72	0.35
Loss on ignition	12.44	13.21	0.77 ± 1.52	0.51	7.04	6.14	0.90 ± 1.58	0.57

Chemical analyses* of the same samples gave a higher average lime requirement for the poor soils than for the good ones. It was also observed that the water-holding capacity was higher and the percolation rate lower in the case of poor soils.

These facts suggested a study of the rate of evaporation of water from soils of the two classes, and also of the effect of lime upon the rate of evaporation.

B. EVAPORATION EXPERIMENTS

For this study, two good and two poor soils were examined. Following the method used by Keen (8), the soil samples were moistened and supported on specially constructed trays in drying chambers where they were weighed periodically to determine the loss of water by evaporation. The apparatus was arranged as in Fig. 1 to enable the temperature of the samples to be kept constant during the experiment. A constant temperature bath *B*, filled with water, was maintained at 25° C. It contained six glass drying chambers *C*, 4½ in. in diameter and 5 in. deep, supported by a wooden lid that covered the bath. These contained 50 cc. of concentrated sulphuric acid and were covered by glass plates made air tight by a vaseline seal. In each drying chamber a soil sample *S* was placed, and the rate of evaporation was determined by weighing the samples at intervals on an analytical balance supported above the constant temperature bath. The latter was on a rotating stand so that any one of the six samples could be weighed without removing it from its drying chamber. During the weighing the aperture in the lid of the drying chamber was only about 5 or 6 sq. mm. in area. The samples were weighed every 15 min. until the weights became approximately constant, after which they were completely dried at 105° C. and the weight of dry soil

* *Chemical analyses were carried out by the Chemistry Department of Macdonald College.*

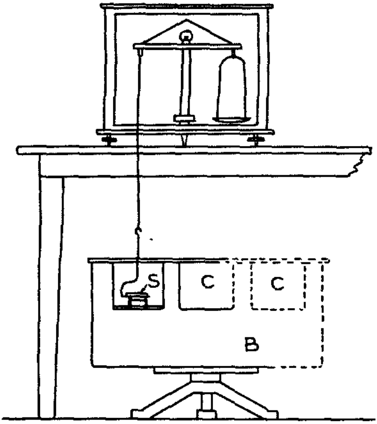

FIG. 1. *Apparatus for the determination of the rate of evaporation of water from the soil (after Keen).*

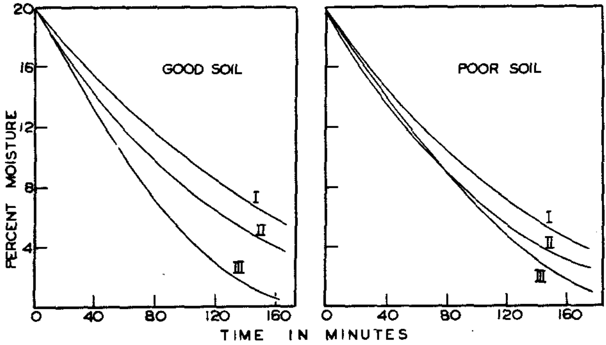

FIG. 2. *Rate of evaporation of water from soils. Curve I shows the rate of loss of moisture from the untreated soil; Curve II is obtained after treatment with limewater; Curve III is obtained after ignition at 700° C. for one hour.*

was determined. The percentage of moisture present at any time could then be calculated and the different soil samples compared.

It was not easy to secure consistent results from duplicate samples. Nevertheless, the general shape of the drying curves may be established. Typical results for one good and one poor soil are shown in Fig. 2.

To study the effect of liming, samples were moistened with limewater and observed as before. The effect of the organic portion of the soil was also studied by observing the rate of evaporation from samples which had been ignited at 700° C. for one hour. Typical results are included in Fig. 2.

The drying curves indicate no significant difference in the rate of evaporation from good and from poor soils. It is, however, clear that treatment with lime hastens the rate at which moisture is lost from the sample; removal of organic matter by ignition produces the same effect. Owing to the variability in the results from different samples it is impossible to say whether liming has a relatively greater effect upon the good or the poor soils; neither is it evident whether the improved crop response, which has been observed upon addition of lime to the poor soils, can be attributed to changes in physical condition.

C. Moisture Equivalent

The rate of evaporation from a soil depends, among other factors, upon the tenacity with which it holds water. To study this property, measurements of moisture equivalent (3) were made upon a number of samples. The moisture equivalent is the percentage of moisture, in terms of dry weight, which a soil can retain when subjected to a force equal to approximately 1,000 times gravity. A special centrifuge is used, fitted with suitable soil cups and adjusted to run at 2440 r.p.m. The method was as follows: a volume of 30 cc. of soil after passing through a 1 mm. sieve, was measured into each centrifuge soil cup. Filter paper was used to cover the perforated sides of the soil cups, and the weight of the filter paper was included with the weight of each empty cup. The samples were moistened from below by allowing the soil cups to stand in distilled water to a depth of 7 or 8 mm. for 15 min. After draining in a covered vessel for 20 to 24 hr., the samples were centrifuged in the machine at a speed of 2440 r.p.m. for 40 min. The samples were then weighed, dried in an electric oven at 105° C., and after cooling in a desiccator were again weighed. The ratio of the moisture retained after centrifuging to weight of dry soil gives the moisture equivalent.

Four good and four poor soils were examined. The average value of moisture equivalent was 26.4% for the former and 30.3% for the latter. Allowing for the deviations among the samples, the difference is not enough to be significant, although it indicates that the poor soils hold water more tenaciously. The samples whose drying curves are shown in Fig. 2 were found to have almost identical values for moisture equivalent: *viz.*, 30.9 and 30.6% for the good and poor samples respectively. Hence, there is no evident relation between moisture equivalent and rate of evaporation for the soils studied.

D. HEAT OF WETTING

It has been known for some time that a measurement of the heat of wetting (2) of a soil offers a means for estimating the proportion of material in the colloidal state. The heat of wetting is the heat, in calories, evolved when 1 gm. of dry soil is wetted with water. This quantity was determined for a number of samples by the method of Janert (7).

For each determination, from 10 to 20 gm. of soil was placed in a glass weighing tube, dried at 105° C. overnight, and the weight of dry soil determined. The stopper was sealed by applying a small quantity of melted paraffin wax, and the sealed weighing tube placed in a Dewar vessel containing enough water to make a total volume of 100 cc. when the soil was added. The Dewar vessel, fitted with a stirrer and a Beckmann thermometer, was closed and allowed to reach a uniform temperature. The weighing bottle was then removed from the water, and, with a minimum of handling, the stopper was removed, the soil poured into the water, and the temperature rise noted. Knowing the water equivalent of the calorimeter (which must be determined separately), the heat of wetting per gram of dry soil can be calculated. Typical results are given in Table III.

TABLE III

HEAT OF WETTING OF QUEBEC SOILS

"Good" soils		"Poor" soils	
Sample No.	Heat of wetting, cal. per gm.	Sample No.	Heat of wetting, cal. per gm.
1a	3.33	7a	3.66
2a	5.09	8a	3.14
3a	2.59	9a	3.07
5a	5.26	10a	3.47
13a	5.28	7b (subsoil)	1.84

By an analysis of variance or by the use of Fisher's *t* table (5) it is found that there is no significant difference between the heat of wetting of the good and poor samples. It is strongly indicated, however, from Tables I and III, that the heat of wetting is correlated with loss on ignition, but is only slightly dependent on the amount of clay in the sample. This point will be examined in Part II which will deal with virgin soils.

E. VOLUME WEIGHT UNDER FIELD CONDITIONS

This experiment was an attempt to detect a change in the volume weight due to heavy lime treatment. The volume weight is defined as the weight of oven-dry soil per unit volume. If expressed in grams per cubic centimeter

it is evidently numerically equal to the apparent specific gravity. If measured in the field, upon undisturbed soil, it is a measure of the compactness.

Various methods have been described to determine the volume weight (6). In these tests, however, the samples were taken and the measurements made with a specially constructed instrument described below.

The soil sampler and its method of use are illustrated in Fig. 3. The sampler is made from steel tubing $3\frac{1}{4}$ in. in diameter, 4 in. long, and of wall thickness $\frac{1}{16}$ in. Two quadrants of the tube are used. These are sharpened, fitted with end plates and handles, and hinged together by means of a $\frac{1}{8}$ in. steel rod. In order to make a measurement, an area about one foot square must be levelled. The open sampler is placed so that it rests upon the levelled ground in the form of a semi-cylinder with convex side up (Fig. 3A). The steel rod which acts as hinge-pin projects about $2\frac{1}{2}$ in. on each side of the sampler. Upon these projections two flat boards 4 by 10 in. are placed. The operator stands upon these boards and, by rotating the handles of the instrument, forces the cutting edges into the ground (Fig. 3B). In this way a semi-cylindrical sample is obtained whose volume is accurately known. With the present instrument the semi-cylinder is 8.4 cm. in diameter, 10.0 cm. long, and has a volume of 277 cc. The sampler containing the soil is weighed upon a spring balance, (Fig. 3C), and samples are taken for moisture determinations, so that the volume weights may be expressed on oven-dry basis.

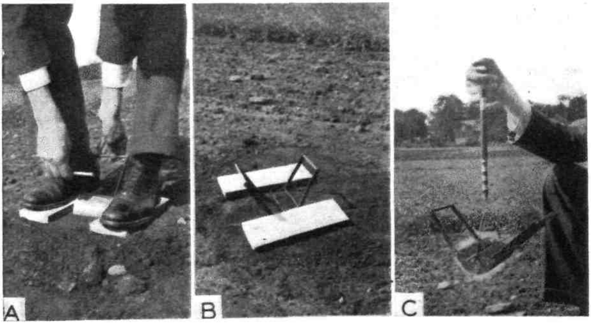

FIG. 3.

Measurements were made at two different locations on the farm of Mr. L. J. Scott, Sawyerville, in Compton Co., Que. At the first location, samples were taken from two neighboring plots, one of which had received, four years before, an application of pulverized limestone at the rate of six tons per acre, while the other had received none. The beneficial effect of lime upon the crop was still evident. Eleven samples were taken from each plot and suffi-

cient moisture determinations were made to reduce weights to oven-dry basis. The results are recorded in Table IV.

TABLE IV

VOLUME WEIGHTS OF LIMED AND UNLIMED SOILS

Limed plot		Unlimed plot	
	Volume weights, gm. per cc.		Volume weights, gm. per cc.
	0.761		0.744
	0.801		0.849
	0.718		0.795
	0.758		0.791
	0.761		0.791
	0.755		0.697
	0.712		0.752
	0.744		0.712
	0.754		0.765
	0.697		0.765
	0.683		0.755
Mean	0.740	Mean	0.765

The difference in volume weights required for significance ($P = 0.05$) is 0.036 gm. per cc.; the difference found, *viz.*, 0.025 gm. per cc., is therefore not significant.

At the second location, on the same farm, similar tests were made on two other plots, one of which had received a recent heavy application of lime. The results again showed no significant difference.

Measurements were also made on limed and unlimed plots at Macdonald College with the same result. Here, however, a difference was not anticipated, as lime produces, in general, no marked change in the crop response.

F. Penetration Experiments

These tests were carried out for the same purpose as the volume weight measurements, *viz.*, to detect possible differences in physical properties due to lime treatment. Keen (9) described experiments in which a metal rod was driven into the ground by repeated blows of a falling weight, the depth of penetration for each succeeding blow being measured and curves plotted to illustrate the rate of penetration.

The apparatus used here was designed to simplify the field readings and to give permanent records. Fig. 4 shows the form of construction. The penetrating portion P consisted of four $\frac{1}{4}$ in. brass rods with square-cut ends. These rods were driven into the ground by a mass M of 1.5 kg. falling through a fixed distance. After each impact the depth of penetration was recorded by a punch-mark on a 3 by 5 in. card C attached to the frame of the device. A number of traces could be made on the same card by sliding it sideways, after which the card was removed from the device and the depth of penetration per impact measured. The apparatus is not satisfactory in stony ground. In fact, pene-

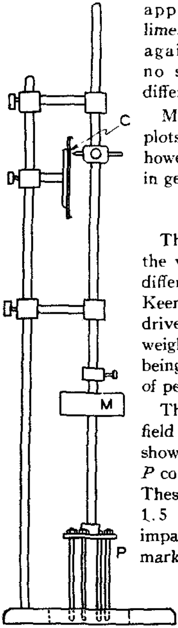

FIG. 4. *Apparatus for measuring the penetration resistance of the soil.*

tration measurements should not be attempted in such soils. A few stones, however, do not interfere with the use of the device because it is usually possible to detect the obstruction by the difference in the sound caused by the impact.

Penetration tests were carried out on two adjacent plots on the farm of Mr. L. J. Scott at Sawyerville. One of these had received a heavy treatment of lime several years before the test. Ten traces were obtained from each plot and these furnish the data for the curves shown in Fig. 5.

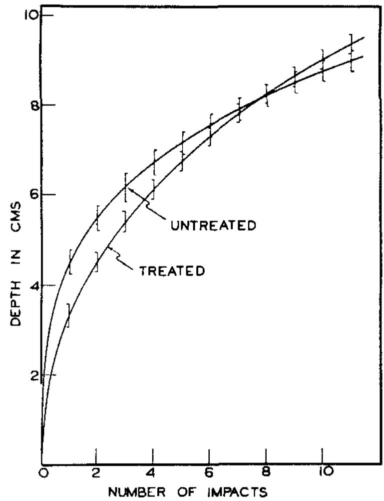

FIG. 5. *Rate of penetration. The vertical lines indicate the probable errors in the determination.*

The curves illustrating the rate of penetration can be represented fairly accurately by equations of the form $y = ax^b$, where y is the depth of penetration and x is the number of impacts. Although the tests were made within a few feet of the line separating the treated from the untreated plot, the possibility of a variation in the soil, other than that caused by the lime, must not be overlooked. Nevertheless, it is suggested that near the surface the treated plot offers significantly more resistance to penetration than the untreated one. This is caused, perhaps, by increased granulation due to the added lime.

G. Apparent Density, Pore Space, Etc., of Laboratory Samples

The apparent difference in the resistance to penetration, mentioned in the previous paragraph, suggested a comparison of several other physical properties. The method of the Keen-Raczkowski "box" experiment (10) enables measurements of apparent and real density, pore space, moisture content in saturated soil, and volume expansion upon wetting to be measured. For this determination, finely pulverized soil is packed into a shallow cylindrical box which has a number of holes bored through the bottom. After the dry soil is weighed it is allowed to reach saturation by taking up water, and as a result the soil swells, thrusting up a dome-shaped extrusion above the top of the box. This is removed with a sharp knife and is used to determine the "volume expansion", *i.e.*, the percentage increase in volume of the saturated soil over the apparent volume of the original soil. The amount of water taken up per unit volume of soil may also be determined as well as the other properties enumerated above. The details of the procedure are given by Coutts (4) or Wright (16) and will not be repeated here. The measurements were made with samples of air-dried soil taken from the same plots that had been used for resistance to penetration and for measurement of volume weight under field conditions as described above. The results of duplicate analyses are given in Table V.

TABLE V

Physical properties of soil by the "box" method

——	Apparent density, gm. per cc.	Real density, gm. per cc.	Pore space, %	Moisture content of sat'd soil, %	Volume exp'n upon wetting, %
Plot A_1 treated	1.04 1.04	2.62 2.60	56.3 56.1	61.5 61.0	9.3 7.8
Plot A_2 untreated	1.04 1.04	2.62 2.65	56.7 56.8	62.8 61.6	10.0 9.1
Plot B_1 treated	0.97 0.97	2.58 2.56	57.8 57.7	66.7 66.6	8.0 9.1
Plot B_2 untreated	1.01 0.99	2.66 2.61	57.6 57.6	63.2 64.8	7.8 8.4

Plot A_1 had received lime at the rate of six tons per acre four years before the samples were taken, and the crop still showed a marked improvement in growth over plot A_2 which had received no lime. Plots B_1 and B_2 were several hundred yards from A_1 and A_2. Plot B_1 had received a heavy application of lime two years before the samples were taken.

It is easily seen from the above figures that heavy lime applications have produced no detectable difference in the physical properties enumerated. This agrees with the absence of significant difference in volume weights under

field conditions. It does not, of course, preclude the possibility of differences in soil structure which must be assumed to account for the apparent difference in resistance to penetration previously mentioned.

Summary

In the surface layers, good alfalfa soils of Missisquoi and Brome Counties have a preponderance of fine gravel, coarse and medium sand; the poor soils have more very fine sand and silt.

In the deeper layers, good alfalfa soils have more fine gravel; the poor soils have more very fine sand and silt.

The effect of lime treatment on the rate of evaporation of water is approximately equal for good and for poor soils.

There is no significant difference in the moisture equivalent or the heat of wetting of good and poor soils, in the few samples studied.

There is evidence that heat of wetting depends to a greater extent on the amount of organic material in these soils than it does upon clay.

Lime treatment produces no significant difference in the volume weight under field conditions, volume weight of laboratory samples, pore space, moisture content of saturated soil, or volume expansion upon wetting, but does, apparently, increase the granulation near the surface as tested by penetration experiments.

Apparatus is described for the measurement of volume weight of soil *in situ* and for the measurement of resistance to penetration.

Acknowledgment

The author is indebted to the Department of Agronomy, Macdonald College, for a classification of the alfalfa fields.

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PHYSICAL PROPERTIES OF MINERAL SOILS OF QUEBEC

PART II. VIRGIN SOILS[1]

By W. Rowles[2]

Abstract

Fifty-three samples from various horizons of virgin soil from different parts of the province have been analyzed for the following properties: organic carbon, loss on ignition, clay, silt, sand, moisture at the sticky point, moisture take up at 50% relative humidity, pore space, heat of wetting, volume expansion, maximum moisture taken up, index of texture, apparent and absolute specific gravity. Correlation coefficients and regression equations have been derived to trace the relations existing between these properties, and it is shown that the organic content of the soil is the most important factor in determining the physical behavior. A comparison of the pipette and hydrometer methods of mechanical analysis is described.

Introduction

Part I of this paper dealt with cultivated soils located, for the most part, in the counties of Missisquoi and Brome, Que. The studies now to be described were of samples from a wider area of Quebec and were carried out to obtain information on soils of which the natural horizons had never been disturbed by cultivation*. At the same time, a comparison of the pipette and hydrometer methods of mechanical analysis was made to test the suitability of the latter method for use in a proposed soil survey.

One of the objects of this study was to obtain data on the texture of the different horizons of various soil types. In addition to the important quality of texture, there are a number of other soil "constants" which are useful, *viz.*, moisture content at the point of stickiness, moisture taken up at 50% relative humidity, maximum water-holding capacity, loss on ignition, volume expansion upon wetting, heat of wetting, pore space, and the apparent and true specific gravities. Some of these constants are known to be related. Numerous attempts have been made to discover a "single-valued constant" that might serve to describe adequately a given soil, and although, as Keen points out, the realization of this hope seems unlikely, the search has furnished much information of value.

In the present work all the above-mentioned constants have been measured, together with some others, and a number of relations have been traced between them. Several types of Quebec mineral soils were studied, namely, those classified as podsol (lowland and upland), brown earth, sandy clay, heavy clay and orchard soil (15). The various horizons were examined separately.

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Contribution from the Department of Physics, Faculty of Agriculture of McGill University, Macdonald College, Que., Canada. Journal Series No. 99.
[2] *Associate Professor of Physics, Macdonald College.*

* *A very brief preliminary report of part of this work has already been published (19).*

Methods of Analysis

A. Mechanical Analysis

(a) Pipette Method

Of the many methods available to determine the texture of a soil, that described by Robinson (18) has been adopted by the International Society of Soil Science. It is based on the assumption that soil particles fall through a viscous medium with a velocity (V) given by the well-known law of Stokes:

$$V = \frac{2g(\sigma - \rho)r^2}{9\eta}$$

where g is the acceleration due to gravity, σ and ρ are the densities of the particle and medium respectively, r is the radius of the equivalent spherical particle, and η is the coefficient of viscosity of the medium.

In practice, the soil sample is thoroughly dispersed in water and the suspension is allowed to stand undisturbed while the particles settle. At definite times and depths, small samples are removed from the suspension by means of a pipette and the amount of solid material in each is determined. From Stokes' Law, the weight of solid material may be expressed as the percentage of silt, clay, etc., in the original sample. The percentage of larger particles, *e.g.*, sand, is more conveniently determined by sieving.

Robinson's method, modified in 1933 (10), was used to determine the percentages of the following International fractions: coarse sand, 2.0–0.2 mm. diameter; fine sand 0.2–0.02 mm.; silt 0.02–0.002 mm.; clay <0.002 mm. In addition to these separates, a determination of clay <0.005 mm. was made by pipetting at the appropriate time and depth. Dispersion was effected by the use of sodium hydroxide. The results are given in Table I.

(b) Hydrometer Method

The method of mechanical analysis described above demands considerable time. To shorten the procedure, Bouyoucos (1, 2) has suggested the use of a special hydrometer which may be placed in the soil suspension, the readings giving a measure of the percentage of silt and clay in the sample. For the preliminary dispersion he recommends an electrically-driven drink mixer with a special paddle. The method has been criticized because of its empirical nature and the variable results which are obtained with some soils due to the disintegration of the particles during vigorous stirring. It was considered desirable to test the applicability of this quick method to Quebec soils.

The procedure here followed was that recommended by Bouyoucos (3), except that additional hydrometer readings were taken at times estimated to give results on the International scale, as well as those required by the American scheme of classification. A partial list of the observed results is included in Table I.

TABLE I

MECHANICAL ANALYSIS OF QUEBEC MINERAL SOILS

Sample number	Name and type o soil	International pipette method								Hydrometer method					
		Horizon	Coarse sand 2.0–0.2 mm.	Fine sand 0.2–0.02 mm.	Silt .02–.002 mm.	Clay .005 mm.	Fineclay .002 mm.	Loss on ignition	$CaCO_3$	Coarse sand (40 sec.)	Total sand (1 min.)	Silt 100 − (total sand + .005 clay)	Clay .005 mm. (1 hr.)	Clay .002 mm. (2 hr.)	Silt and clay (5 min.)
	BROWN EARTH														
1	Cowansville	B	39.1	25.4	20.8	17.4	11.1	6.84	0	54.5	58.5	26.7	14.8	8.9	35.3
2	Shefford	B	59.5	13.5	12.0	12.3	9.9	6.60	0	62.7	64.8	25.5	9.7	5.2	26.8
3	Summerlea	A	19.4	32.5	16.6	19.2	14.8	14.70	0	39.9	47.3	39.7	13.0	11.1	32.8
4	Summerlea	B	26.5	36.0	15.7	19.7	14.1	8.14	0	46.6	50.8	36.7	12.5	9.8	31.0
5	Summerlea	C	26.3	39.7	16.3	18.5	13.7	5.07	0	46.9	51.0	34.5	14.5	12.1	32.4
6	Eastman	A	28.6	25.1	18.0	17.2	12.6	14.44	0	35.8	37.8	28.0	34.2	29.8	43.6
7	Eastman	B	41.7	29.1	12.6	12.9	9.3	7.26	0	57.8	59.9	28.9	11.2	6.6	28.6
8	Eastman	C	26.5	40.9	20.2	17.8	11.7	1.78	0	53.2	56.3	27.9	15.8	12.4	32.7
9	Morgan's Wood (Senneville)	A	16.1	27.8	18.1	29.5	21.4	19.53	0.56	39.2	41.5	46.3	12.2	10.6	43.1
10	Morgan's Wood (Senneville)	B	14.3	41.4	26.4	27.4	16.6	5.30	1.84	31.3	33.1	40.3	26.6	19.7	45.0
11	Morgan's Wood (Senneville)	C	8.6	52.0	27.7	17.0	9.1	4.36	2.24	27.9	32.2	52.7	15.1	10.8	41.9
	UPLAND PODSOL														
12	Stukeley	A_2	30.6	38.9	21.5	12.9	7.7	2.91	0	54.2	56.7	32.5	10.8	8.4	28.2
13	Stukeley	B_1	37.8	27.8	15.0	11.8	8.5	13.57	0	50.9	56.3	36.3	7.4	5.2	20.8
14	Stukeley	B_2	37.9	33.9	15.3	11.5	7.8	6.27	0	56.0	58.7	31.7	9.6	7.5	28.7
15	Stukeley	C	37.2	39.1	14.6	14.6	10.3	2.55	0	58.5	62.2	27.2	10.6	7.1	27.2
16	Ste. Adele	A_1	2.6	44.8	16.0	17.7	11.1	30.88	0.82	48.7	54.2	35.9	9.9	7.9	20.4
17	Ste. Adele	A_2	3.1	77.6	14.1	12.8	9.1	1.92	0	50.8	55.9	37.3	6.8	6.4	16.0
18	Ste. Adele	B_1	2.5	62.3	11.4	11.9	9.9	19.25	0	51.3	61.8	31.0	7.2	6.3	10.3
19	Ste. Adele	B_2	3.6	65.3	13.1	11.3	8.5	7.78	0	56.3	63.9	30.2	5.9	5.0	11.3
20	Ste. Adele	C	20.6	57.8	9.5	8.3	5.3	3.13	0	66.2	70.3	24.3	5.4	4.9	11.5

	SANDY CLAY													
21	Marieville	B_1	24.0	29.4	18.4	31.2	23.9	3.17	0	45.5	50.7	19.6	29.7	21.7	40.0
22	Marieville	B_2	34.8	38.3	13.2	12.9	9.7	1.55	1.48	65.0	69.0	22.1	8.9	6.7	18.9
23	Marieville	C	54.3	14.4	15.9	16.3	10.6	1.33	2.83	61.4	67.5	23.1	9.4	7.4	24.5
24	Pike River	A	1.6	47.1	21.8	29.9	24.5	11.20	0	35.5	40.7	43.1	16.2	14.1	37.4
25	Pike River	B_2	3.2	52.2	17.4	30.8	27.3	2.86	0	36.8	42.8	26.2	31.0	28.9	39.8
26	Pike River	C	4.8	15.6	27.4	64.8	50.0	3.27	0	14.4	16.5	19.3	64.2	55.6	78.5
27	Laprairie	A	11.3	15.4	22.2	45.2	36.0	16.43	0	16.4	18.6	72.9	8.5	6.4	60.7
28	Laprairie	B	29.3	27.3	16.1	26.6	21.2	3.87	0.25	44.4	46.5	24.3	29.2	25.2	40.4
29	Laprairie	C	3.9	59.3	28.3	11.1	6.7	1.34	0.25	29.9	35.9	52.2	11.9	7.3	36.1
30	St. Cuthbert	A	17.6	41.8	17.6	22.0	16.1	8.56	0.43	35.5	38.1	45.1	16.8	13.8	37.6
31	St. Cuthbert	B	23.2	35.0	19.0	25.9	18.6	4.65	0.40	27.2	29.8	33.5	36.7	31.6	53.1
32	St. Cuthbert	C	30.8	16.2	25.0	36.8	25.8	3.94	0.47	10.0	12.3	29.6	58.1	51.4	78.1
33	Three Rivers	A	3.0	26.8	38.1	36.5	22.7	10.52	0.80	21.3	23.9	41.5	34.6	24.0	58.7
34	Three Rivers	B	1.5	25.1	39.6	41.6	25.8	7.95	0.70	17.7	20.4	41.7	37.9	27.2	65.7
35	Three Rivers	C	0.6	25.3	44.9	43.1	27.1	2.89	0.40	10.8	12.8	44.9	42.3	33.8	69.2
	HEAVY CLAY													
36	St. Lin	A	7.3	36.2	18.6	35.8	30.1	8.44	0.24	24.5	29.4	38.2	32.4	29.5	50.3
37	St. Lin	B	11.6	15.5	17.4	58.3	51.7	4.72	0.37	9.2	10.4	21.1	68.5	63.8	81.8
38	St. Lin	C	6.8	8.0	18.4	73.0	63.3	3.26	0.66	6.9	8.2	15.4	76.4	70.9	87.3
39	Laprairie	A	3.8	5.1	20.0	71.5	58.5	15.98	0	15.4	17.5	24.8	57.7	51.6	74.8
40	Laprairie	B	1.9	2.5	24.6	85.9	64.4	5.30	0.25	7.8	8.7	9.2	82.1	76.3	91.3
41	Laprairie	C	5.1	9.5	30.6	63.5	47.5	4.00	1.22	14.3	16.6	19.1	64.3	55.6	78.2
42	Macdonald College	A	0	0	17.1	85.8	78.9	4.99	0.68	2.8	2.8	14.3	82.9	76.1	92.6
43	Macdonald College	B_1	0	0	19.3	85.9	78.7	4.18	0	5.7	5.7	11.4	82.9	76.1	92.8
44	Macdonald College	B_2	0	0	17.6	84.0	75.8	4.34	1.61	6.7	6.7	14.5	78.8	73.1	91.8
45	Macdonald College	C	0	0	20.9	80.3	68.6	5.56	3.96	7.7	7.7	15.6	76.7	70.0	91.3
46	Low	A	0.4	40.8	38.3	23.4	16.4	1.42	0.24	11.4	15.3	68.4	17.3	15.4	52.4
47	Low	B	2.7	15.8	33.1	58.1	44.0	1.78	0.35	9.0	11.3	35.3	53.4	48.4	75.3
48	Low	C	1.3	4.9	34.1	73.8	46.0	1.67	0.45	3.6	5.5	26.2	68.3	62.2	90.1
	ORCHARD SOIL													
49	St. Hilaire	A	28.6	19.3	16.1	21.9	17.7	12.84	0	48.9	52.1	26.2	21.7	21.7	41.2
50	St. Hilaire	B	54.0	18.4	12.7	14.7	11.3	6.43	0	58.9	61.5	29.9	8.6	11.1	26.8
51	Frelighsburg	A	21.4	36.1	22.9	18.7	10.6	9.28	0	45.2	48.0	42.9	9.1	11.5	35.9
52	Frelighsburg	B	30.0	39.3	19.6	12.7	6.6	3.70	0	51.7	56.9	37.4	5.7	9.7	28.9
53	Frelighsburg	C	32.8	42.0	19.7	11.1	5.2	1.74	0	58.2	61.2	33.1	5.7	8.6	26.7

TABLE II

PHYSICAL ANALYSES OF TYPICAL MINERAL SOILS OF QUEBEC

(Sample numbers correspond with Table I)

Symbol	A	B	C	D	E	F	G	H	J	K	L	M	N	P	Q
Sample No.	Organic carbon, %	Loss on ignition, %*	Clay .002 mm., %	Silt .02–.002 mm., %	Moisture at sticky point, %	Moisture at 50% relative humidity, %	Pore space, %	Heat of wetting, cal./gm.	Volume expansion, %	Max. moisture taken up*	Loss on ignition, inorganic (B – 1.724A)	Index of texture	Moisture in air-dry soil	Apparent specific gravity	Absolute specific gravity
BROWN EARTH															
1	2.51	6.84	11.1	20.8	33.2	1.62	54.0	3.31	8.4	53.8	2.51	23.2	3.05	1.12	2.29
2	2.19	6.60	9.9	12.0	31.0	2.61	49.2	3.08	14.8	43.8	2.82	16.4	2.74	1.32	2.27
3	9.05	14.70	14.8	16.6	76.2	4.85	63.8	7.38	25.0	122.8	0.00	65.8	4.81	0.71	1.67
4	3.09	8.14	14.1	15.7	40.1	3.77	54.3	4.30	16.2	60.4	2.81	27.6	4.16	1.12	2.13
5	1.49	5.07	13.7	16.3	28.2	2.76	52.7	2.88	9.5	52.5	2.51	15.0	3.68	1.13	2.23
6	7.45	14.44	12.6	18.0	56.0	3.68	61.4	5.19	13.0	82.6	1.58	45.3	3.57	0.87	2.03
7	3.13	7.26	9.3	12.6	34.8	3.34	56.2	4.08	10.6	61.2	1.86	20.6	2.61	1.04	2.16
8	0.17	1.78	11.7	20.2	14.6	0.62	41.8	0.64	4.8	26.4	1.49	1.1	0.46	1.52	2.54
9	10.37	19.53	21.4	18.1	74.9	4.21	65.1	10.90	30.6	119.2	1.68	66.1	13.22	0.82	1.69
10	2.84	5.30	16.6	26.4	40.8	3.65	60.1	9.41	17.1	72.9	0.40	29.7	7.26	1.02	2.12
11	0.44	4.36	9.1	27.7	43.5	4.55	63.1	12.61	15.0	73.4	3.60	31.4	7.98	1.05	2.38
UPLAND PODSOL															
12	1.16	2.91	7.7	21.5	39.8	0.96	46.3	1.74	5.5	40.7	0.91	25.9	1.25	1.23	2.22
13	5.94	13.57	8.5	15.0	51.7	4.36	61.7	7.10	8.8	75.8	3.33	38.6	8.85	0.93	2.17
14	2.06	6.27	7.8	15.3	32.9	2.31	53.3	3.35	11.7	51.2	2.73	18.5	4.31	1.19	2.25
15	0.66	2.55	10.3	14.6	23.3	1.02	43.0	1.87	9.9	35.3	1.41	8.0	1.56	1.41	2.39
16	20.11	30.88	11.1	16.0	158.6	8.44	66.8	11.10	24.2	221.0	0.00	149.1	9.90	0.42	1.05
17	1.00	1.92	9.1	14.1	34.5	0.60	52.2	0.76	7.0	47.5	0.20	18.4	0.57	1.18	2.37
18	6.30	19.25	9.9	11.4	77.0	8.13	63.5	6.79	11.4	99.4	8.37	66.0	8.83	0.73	1.93
19	2.96	7.78	8.5	13.1	52.5	5.16	60.7	4.23	11.8	71.8	2.68	38.7	5.39	1.05	2.24
20	1.09	3.13	5.3	9.5	30.2	2.63	33.8	2.22	12.0	31.4	1.25	14.5	2.45	1.26	1.75

Sandy Clay														
21	0.37	3.17	23.9	18.4	23.2	1.59	47.9	4.04	7.5	39.0	2.53	12.5	3.06	1.34	2.39
22	0.14	1.55	9.7	13.2	14.0	0.32	35.7	1.54	5.5	23.2	1.31	0.0	0.58	1.64	2.46
23	0.08	1.33	10.6	15.9	14.0	0.37	36.9	0.64	3.9	24.0	1.19	0.3	0.47	1.56	2.43
24	5.59	11.20	24.5	21.8	53.6	2.99	60.7	4.62	17.9	80.1	1.52	43.9	3.88	0.92	2.00
25	0.17	2.86	27.3	17.4	25.4	1.58	49.4	2.58	13.7	47.1	2.57	14.3	3.04	1.26	2.25
26	0.06	3.27	50.0	27.4	32.7	2.97	57.1	4.63	14.7	56.5	3.17	28.6	4.45	1.19	2.49
27	6.81	16.43	36.0	22.2	67.2	5.93	64.7	6.34	14.2	106.6	4.71	61.9	6.78	0.74	1.79
28	0.65	3.87	21.2	16.1	25.9	2.39	50.4	2.90	12.3	45.0	2.75	14.4	4.19	1.30	2.29
29	0.22	1.34	6.7	28.3	21.0	0.04	44.4	1.37	4.3	30.6	0.96	8.4	0.26	1.49	2.64
30	3.70	8.56	16.1	17.6	48.4	3.20	54.9	4.26	12.9	61.5	2.18	36.5	3.73	1.02	1.98
31	1.16	4.65	18.6	19.0	37.4	3.36	54.4	2.38	12.0	56.9	2.65	25.8	3.63	1.12	2.22
32	0.00	3.94	25.8	25.0	37.9	3.60	53.2	4.04	7.0	55.2	3.94	28.5	4.55	1.10	2.20
33	4.93	10.52	22.7	38.1	54.3	3.71	57.5	5.00	14.4	70.8	2.00	48.3	3.62	0.97	2.06
34	3.24	7.95	25.8	39.6	51.8	3.23	53.5	4.14	10.4	62.7	2.35	46.5	3.12	1.00	2.18
35	0.37	2.89	27.1	44.9	30.0	2.00	52.2	2.47	6.4	44.1	2.25	24.8	1.87	1.20	2.36
Heavy Clay															
36	2.47	8.44	30.1	18.6	43.0	4.17	56.0	4.08	13.4	59.2	4.18	34.3	4.57	1.13	2.24
37	1.12	4.72	51.7	17.4	46.2	4.93	59.5	5.11	11.1	67.3	2.79	40.8	5.44	1.07	2.34
38	0.80	3.26	63.3	18.4	48.4	4.96	61.0	5.71	17.7	64.8	1.88	45.4	5.20	1.17	2.54
39	7.49	15.98	58.5	20.0	57.3	4.80	62.4	6.56	13.5	80.2	3.07	55.5	8.20	0.94	2.11
40	3.27	5.30	64.4	24.6	40.0	3.71	54.6	5.04	13.8	57.9	0.00	39.1	6.35	1.16	2.19
41	1.02	4.00	47.5	30.6	31.3	2.48	53.9	3.46	10.3	51.9	2.24	28.4	3.70	1.21	2.31
42	3.00	4.99	78.9	17.1	39.8	3.73	53.1	5.79	19.6	56.9	0.00	39.8	5.49	1.19	2.11
43	2.07	4.18	78.7	19.3	33.9	3.60	56.2	6.78	23.1	61.2	0.61	33.9	5.46	1.19	2.20
44	1.43	4.34	75.8	17.6	40.2	3.29	57.9	5.04	20.9	55.2	1.88	40.2	4.70	1.20	2.26
45	0.57	5.56	68.6	20.9	38.2	2.75	56.9	4.78	19.6	62.2	4.57	38.2	4.00	1.13	2.21
46	0.31	1.42	16.4	38.3	24.5	0.95	46.5	1.34	8.2	35.6	0.89	16.3	0.75	1.36	2.37
47	0.07	1.78	44.0	33.1	33.4	2.62	53.0	2.88	16.0	51.1	1.66	29.7	1.90	1.20	2.56
48	0.00	1.67	46.0	34.1	37.3	2.96	55.8	3.25	18.5	62.5	1.67	36.1	2.46	1.16	2.34
Orchard Soil															
49	6.10	12.84	17.7	16.1	56.8	3.53	57.5	5.61	23.9	65.2	2.32	47.2	4.50	0.97	1.88
50	2.22	6.43	11.3	12.7	33.3	2.14	52.8	2.91	17.5	52.8	2.60	18.8	2.46	1.14	2.14
51	3.89	9.28	10.6	22.9	47.8	2.10	60.4	3.66	7.4	76.8	2.56	36.3	3.07	0.86	2.06
52	1.56	3.70	6.6	19.6	28.9	1.23	49.9	1.55	8.0	42.4	1.01	15.0	1.65	1.28	2.38
53	0.27	1.74	5.2	19.7	19.4	0.43	40.8	0.60	2.2	25.9	1.27	4.4	0.38	1.54	2.53

* *Figures for some Quebec podsol soils have been given by Gray and McMaster (6) and Gray and Taylor (7).*

B. Moisture at the Sticky Point

This measurement, originally advocated by Hardy (8), was determined by adding sufficient water to the dry soil so that, upon thorough kneading, the soil was not quite sticky, but could be rolled out into a cylinder about 2 mm. in diameter without breaking up. After some practice the point can be judged quite accurately. The moist soil was weighed, dried at 105° C. overnight, and weighed again. The "sticky point" is expressed as the percentage of water in the moist soil, on the basis of oven-dry soil weight. As it cannot be determined for sandy soils, Olmstead (16) has suggested the use of minimum water of saturation. This "constant" has been measured for a number of Quebec soils but will not be included here. The results for the percentage of moisture at the sticky point are given in Table II; from these values and the percentage of sand it is possible to calculate the index of texture as proposed by Hardy (9), who defined index of texture as the percentage of moisture at the sticky point minus one-fifth of the percentage of sand. This is also included in the table.

C. Heat of Wetting

The heat of wetting is defined as the heat in calories evolved when one gram of dry soil is wetted with water. It was measured by the method of Janert (11), briefly outlined in Part I.

D. Moisture Taken Up at 50% Relative Humidity

The hygroscopic coefficient formerly in use expressed the amount of water that a soil would take up from a saturated atmosphere. This has gradually been replaced by a determination of the amount of moisture taken up from an atmosphere kept at 50% relative humidity. The measurement has been proposed (5) as a means of estimating the amount of colloidal material in soil.

To make the measurement, samples were placed in weighing dishes in a vacuum desiccator, over sulphuric acid diluted to specific gravity of 1.329 at 25° C. The air pressure in the desiccator was reduced to a few centimeters of mercury and the system allowed to stand at reasonably constant temperature to reach equilibrium. The final constant weight of each sample was observed, after which it was dried at 105° C. overnight, and again weighed. The result is expressed as the percentage of moisture taken up by oven-dry soil.

E. Apparent and Real Specific Gravity, Maximum Moisture-holding Capacity, Pore Space, and Volume Expansion upon Wetting

All the above-mentioned properties may be measured in a single routine, usually known as the Keen-Raczkowski "box" experiment (14), outlined in Part I. The results of the determination on Quebec soils are presented in Table II.

F. Organic Carbon and Loss on Ignition

Loss on ignition was determined in the usual way by ignition at 700° C. for one hour; organic carbon was determined by the dry combustion method described by Read (17).

Experimental Results

In this section, three tables are given to include the more useful data from the numerous analyses that have been made. Tables I and II are self-explanatory; Table III is arranged for easy comparison of the two methods of mechanical analysis. The analyses were all carried out in duplicate, mean values being recorded in the tables. Through unforeseen circumstances it was impossible to make determinations of all the physical properties of every horizon of each soil studied. For convenience in the mathematical work it was desirable to use only those samples for which complete data were available. For this reason, much useful data which were obtained in the analyses have been omitted from the tables, and only those samples which were used in the calculations have been included.

TABLE III

Comparison of mechanical analyses by international pipette method and hydrometer method

No.	Soil type and location	Horizon	Clay (.005 mm.)		Clay (.002 mm.)		Silt and fine clay	
			Pipette method:	Hydrometer method: (1 hr.)	Pipette method:	Hydrometer method: (2 hr.)	Pipette method:	Hydrometer method: (5 min.)
	Brown Earth							
1	Cowansville	B	17.4	14.8	11.1	8.9	31.9	35.3
2	Shefford	B	12.3	9.7	9.9	5.2	21.9	26.8
3	Summerlea	A	19.2	13.0	14.8	11.1	31.4	32.8
4	Summerlea	B	19.7	12.5	14.1	9.8	29.8	31.0
5	Summerlea	C	18.5	14.5	13.7	12.1	30.0	32.4
6	Eastman	A	17.2	34.2	12.6	29.8	30.6	43.6
7	Eastman	B	12.9	11.2	9.3	6.6	21.9	28.6
8	Eastman	C	17.8	15.8	11.7	12.4	31.9	32.7
9	Senneville	A	29.5	12.2	21.4	10.6	39.5	43.1
10	Senneville	B	27.4	26.6	16.6	19.7	43.0	45.0
11	Senneville	C	17.0	15.1	9.1	10.8	36.8	41.9
	Upland Podsol							
12	Stukeley	A_2	12.9	10.8	7.7	8.4	29.2	28.2
13	Stukeley	B_1	11.8	7.4	8.5	5.2	23.5	20.8
14	Stukeley	B_2	11.5	9.6	7.8	7.5	23.1	28.7
15	Stukeley	C	14.6	10.6	10.3	7.1	24.9	27.2
16	Ste. Adele	A_1	17.7	9.9	11.1	7.9	27.1	20.4
17	Ste. Adele	A_2	12.8	6.8	9.1	6.4	23.2	16.0
18	Ste. Adele	B_1	11.9	7.2	9.9	6.3	21.3	10.3
19	Ste. Adele	B_2	11.3	5.9	8.5	5.0	73.8	11.3
20	Ste. Adele	C	8.3	5.4	5.3	4.9	63.1	11.5

TABLE III—*Concluded*

COMPARISON OF MECHANICAL ANALYSES BY INTERNATIONAL PIPETTE METHOD AND HYDROMETER METHOD—*Concluded*

No.	Soil type and location	Horizon	Clay (.005 mm.)		Clay (.002 mm.)		Silt and fine clay	
			Pipette method:	Hydrometer method: (1 hr.)	Pipette method:	Hydrometer method: (2 hr.)	Pipette method:	Hydrometer method: (5 min.)
	SANDY CLAY							
21	Marieville	B_1	31.2	29.7	23.9	21.7	42.3	40.0
22	Marieville	B_2	12.9	8.9	9.7	6.7	22.9	18.9
23	Marieville	C	16.3	9.4	10.6	7.4	26.5	24.5
24	Pike River	A	29.9	16.2	24.5	14.1	46.3	37.4
25	Pike River	B_2	30.8	31.0	27.3	28.9	44.7	39.8
26	Pike River	C	64.8	64.2	50.0	55.6	77.4	78.5
27	Laprairie	A	45.2	8.5	36.0	6.4	58.2	60.7
28	Laprairie	B	26.6	29.2	21.2	25.2	37.3	40.4
29	Laprairie	C	11.1	11.9	6.7	7.3	35.0	36.1
30	St. Cuthbert	A	22.0	16.8	16.1	13.8	33.7	37.6
31	St. Cuthbert	B	25.9	36.7	18.6	31.6	37.6	53.1
32	St. Cuthbert	C	36.8	58.1	25.8	51.4	50.8	78.1
33	Three Rivers	A	36.5	34.6	22.7	24.0	60.8	58.7
34	Three Rivers	B	41.6	37.9	25.8	27.2	65.4	65.7
35	Three Rivers	C	43.1	42.3	27.1	33.8	72.0	69.2
	HEAVY CLAY							
36	St. Lin	A	35.8	32.4	30.1	29.5	48.7	50.3
37	St. Lin	B	58.3	68.5	51.7	63.8	69.1	81.8
38	St. Lin	C	73.0	76.4	63.3	70.9	81.7	87.3
39	Laprairie	A	71.5	57.7	58.5	51.6	78.5	74.8
40	Laprairie	B	85.9	82.1	64.4	76.3	89.0	91.3
41	Laprairie	C	63.5	64.3	47.5	55.6	78.1	78.2
42	Macdonald College	A	85.8	82.9	78.9	76.1	96.0	92.6
43	Macdonald College	B_1	85.9	82.9	78.7	76.1	93.0	92.8
44	Macdonald College	B_2	84.0	78.8	75.8	73.1	93.4	91.8
45	Macdonald College	C	80.3	76.7	68.6	70.0	89.5	91.3
46	Low	A	23.4	17.3	16.4	15.4	54.7	52.4
47	Low	B	58.1	53.4	44.0	48.4	77.1	75.3
48	Low	C	73.8	68.3	46.0	62.2	90.1	90.1
	ORCHARD SOIL							
49	St. Hilaire	A	21.9	21.7	17.7	21.7	33.8	41.2
50	St. Hilaire	B	14.7	8.6	11.3	11.1	24.0	26.8
51	Frelighsburg	A	18.7	9.1	10.6	11.5	33.5	35.9
52	Frelighsburg	B	12.7	5.7	6.6	9.7	26.2	28.9
53	Frelighsburg	C	11.1	5.7	5.2	8.6	24.9	26.7

Discussion

COMPARISON OF PIPETTE AND HYDROMETER METHODS OF MECHANICAL ANALYSIS

From an examination of the data in Table III it may be seen that there is considerable variation between the results of the two methods of analysis. This is more evident for the smaller particle sizes of the 0.005 mm. and 0.002 mm. clays; it is less serious in the case of the larger separates which contain

silt. It is not easy to state unequivocally whether the hydrometer may safely be used to replace the pipette method, because the fair agreement which is generally obtained is seriously marred in some cases by very large discrepancies. For example, in the *A* horizon of Laprairie sandy clay (Sample No. 27), the values obtained for the 0.005 mm. clay are 45.2 and 8.5% respectively. It is unlikely that this is an error in observation as a similar difference was obtained in the analysis of 0.002 mm. clay for this sample, *viz.*, 36.0 and 6.4% respectively. The difference is evidently attributable to the difference in the two methods of analysis.

The advisability of examining the results statistically has been considered, but in view of the fact that there are occasional wide variations and because of the difficulty which these would introduce into the interpretation, it seemed best not to attempt such an analysis.

In seeking to describe soil samples accurately, the value of a method may well be inversely proportional to the chance of making a large error in the analysis. With this limitation, it is of no avail that most of the analyses be highly accurate if the remainder be very inaccurate. It is open to question whether this is true in the study of soil properties. Suffice it to say that on the basis of results obtained with Quebec soils, the hydrometer method usually gives reasonably accurate results, but, without doubt, very large errors may sometimes occur.

Relations Between Soil Constants

It is well known that some of the soil properties enumerated above are dependent on one another. Keen and Coutts (13) and Keen (12) have studied the relations between the sticky point, the loss on ignition, the moisture content in equilibrium with an atmosphere of 50% relative humidity, and the percentage clay content of the soil. From their work it appears that "the sticky point is largely controlled by the material in the soil that is driven off by ignition, while the soil moisture content at 50% relative humidity is controlled largely by the clay content."

Upon analyzing the results for Quebec soils, interesting facts appear, not all of which are in agreement with the results obtained for the soils examined by Keen and Coutts.

Scatter diagrams (not shown) of certain pairs of variables for the 53 samples already described indicate strong association in most cases, but little or none where clay is concerned. A calculation of the same simple and partial correlations that were given by Keen and Coutts for their original 39 samples, and later for the 250 soils from various parts of the world, shows that in some respects the Quebec samples depart significantly from the general behavior. Using, for the moment, Keen's notation in which C is the clay content, R is the moisture content in equilibrium with an atmosphere of 50% relative

humidity, I the loss on ignition, and S the moisture at the sticky point, the values of correlation coefficients, with their standard errors, are as follows:

TABLE IV

CORRELATION COEFFICIENTS (SIMPLE)

	Data for original 39 soils (Keen and Coutts)			*Data for 250 soils (Keen)*			Data for 53 *Quebec* soils		
	I	R	S	I	R	S	I	R	S
C	.364	.719	.317	.283 ± .060	.483 ± .050	.430 ± .053	−.108 ± .138	.231 ± .133	.004 ± .140
I	—	.388	.865	—	.632 ± .039	.820 ± .021	—	.772 ± .057	.913 ± .024
R	—	—	.303	—	—	.670 ± .036	—	—	.830 ± .044

It may be observed that for Quebec soils none of the coefficients involving clay is significant, while all the others are highly significant and definitely larger than those previously reported.

A further comparison is possible by the use of partial correlation coefficients which are given in the following table, together with Keen's data.

TABLE V

PARTIAL CORRELATION COEFFICIENTS

—	Data for 39 soils (Keen and Coutts)	Data for 250 soils (Keen)	Data for 53 Quebec soils
SI.C	0.843	0.808 ± 0.023	0.919*
RC.I	.673	.410 ± .054	.340*
RI.C	.194	.590 ± .042	.824*
SC.I	.155	.362 ± .054	.252
RI.S	.095	.193 ± .063	.062
SC.R	.015	.163 ± .063	−.346*

*For the Quebec soils, values marked * are significant.*

The first and third of the significant values are higher than have previously been found, while the last exhibits most unexpected behavior in that it is negative. This is so different from the result found by Keen that it seemed wise to examine the data in greater detail, taking more variables into consideration. These extended calculations were made by the method of Wallace and Snedecor (20). When each variable is considered with respect to each of the others, 59 simple correlation coefficients are obtained, as shown in Table VI.

TABLE VI

CORRELATION COEFFICIENTS BETWEEN VARIOUS SOIL "CONSTANTS" OF 53 QUEBEC SOILS

Column I contains the simple correlation coefficients; column II gives the partial correlation coefficients. The variables are as follows:

A Organic carbon, %
B Loss on ignition, %
C Clay, %
D Silt, %
E Moisture content at the sticky point, %
F Moisture taken up at 50% relative humidity, %
G Pore space, %
H Heat of wetting, cal. per gm.
J Volume expansion, %
K Maximum moisture taken up, %
L = B − 1.724A
M Index of texture
* Significant, $P = 0.05$; $r > 0.295$
** Highly significant, $P = 0.01$; $r > 0.381$

Pair	I	II	Pair	I	II	Pair	I	II
AB	.961	.860**	CG	.272	± .119	GJ	.609	.295*
AC	−.104	.229	CH	.211	.210	GK	.768	.386**
AD	−.193	−.131	CJ	.426	.431**	HJ	.689	.362*
AE	.928	.404**	CK	.034	−.027	HK	.763	.290
AF	.688	−.616**	DE	−.092	.285	JK	.614	± .037
AG	.612	−.080	DF	−.124	−.331*	MA	.885	—
AH	.642	.196	DG	.062	.290	MB	.872	—
AJ	.535	.120	DH	−.025	.178	MC	.197	—
AK	.918	.164	DJ	−.113	± .113	MD	.010	—
BC	−.108	−.333*	DK	−.084	.081	ME	.979	—
BD	−.214	−.029	EF	.830	.624**	MF	.841	—
BE	.913	−.196	EG	.714	−.188	MG	.756	—
BF	.772	.543**	EH	.706	−.356*	MH	.730	—
BG	.685	.387**	EJ	.566	.038	MJ	.629	—
BH	.667	− 165	EK	.972	.700**	LA	−.053	—
BJ	507	− 094	FG	.795	.136	LC	− 024	—
BK	.900	− 112	FH	.775	.348*	LD	−.092	—
CD	.208	.256	FJ	.572	−.118	LF	− 386	—
CE	.004	± .165	FK	.812	−.182	LG	.292	—
CF	.231	.407**	GH	.778	± .058			

The values of the simple correlation coefficients in Table VI range from the high value of 0.979, which is the simple correlation between moisture content at the sticky point and the index of texture, to negligibly small or non-significant values. In 12 pairs of variables, r is greater than 0.800, and in seven of these, r is greater than 0.900.

It is clear that some of these high-correlations cannot be attributed to any causal relation between the two variables in question, but rather to dependence upon one or more other variables. To study this dependence in more detail, the partial correlations have been calculated for all the variables except L and M. The partial correlation coefficients, also given in Table VI, bring out the extent of the association between each pair of variables independent of the accompanying variation in the other variables. As tested by the usual criterion for significance, 16 coefficients are found to be significant, and of these, ten are highly significant.

It will be observed that the partial correlation coefficient between sticky point and clay, after elimination of the effects of the other variables, is now found to be non-significant. It is apparent, therefore, that the significant

negative correlation given in Table V was due to the fact that an insufficient number of variables had been used in the calculation.

The variable showing significant partial correlation with the greatest number of others is the moisture taken up at 50% relative humidity. This property is correlated positively with the percentage of organic carbon, the percentage of clay, the moisture at the sticky point, and the heat of wetting. It is negatively correlated with the loss on ignition and the percentage of silt. Table VII has been prepared to illustrate the relative number of soil properties showing significant partial correlations with each other.

TABLE VII

SOIL PROPERTIES WHICH SHOW SIGNIFICANT PARTIAL CORRELATIONS WITH EACH OTHER

Variable	Positive correlations	Negative correlations
Organic carbon, %, (A)	B, E	F
Loss on ignition, %, (B)	A, F, G	C
Clay, %, (C)	F, J	B
Silt, %, (D)	—	F
Moisture content at the sticky point, %, (E)	A, F, K	H
Moisture taken up at 50% relative humidity, %, (F)	B, C, E, H	A, D
Pore space, %, (G)	B, J, K	—
Heat of wetting, cal. per gm. (H)	F, J	E
Volume expansion, %, (J)	C, G, H	—
Maximum moisture taken up, %, (K)	E, G	—

On the basis of the facts shown in Tables VI and VII, it seems reasonable to select, as a first approximation, organic carbon, loss on ignition, clay, and silt as independent variables, the others being dependent upon these and possibly other properties, at present undefined. As there is strong association between organic carbon and loss on ignition (partial correlation 0.860), it is doubtful whether both these should be considered as independent variables. Moreover, upon calculating the regression equation for loss on ignition (B) as a function of organic carbon (A), clay (C), and silt (D), the following expression is obtained:

$$B = 1.50A - 0.00123C - 0.0220D + 3.17$$

in which the coefficient of A is highly significant and contributes more than 96% of the total variation in B. For this reason, regression equations have been worked out for the other soil properties in terms of (i) organic carbon, clay, and silt, (ii) loss on ignition, clay, and silt, and (iii) organic carbon, loss on ignition, clay, and silt. These are included in the first 19 equations of Table VIII.

For each of the regression equations just mentioned, the multiple correlation coefficient (R) has been calculated, and the value included in Table VIII. It may be observed that these coefficients are comparatively large, *i.e.*, the

equations predict the values of the independent variables with good accuracy. Furthermore, it appears that the three soil properties, clay, silt, and organic carbon, (or loss on ignition), when used as independent variables, will generally produce almost as high a value of *R* as the inclusion of eight or nine variables. In other words, the three properties mentioned are the important properties and they determine all the others to a very large extent.

TABLE VIII

REGRESSION EQUATIONS FOR PHYSICAL PROPERTIES OF QUEBEC SOILS

A	Organic carbon, %	G	Pore space, %
B	Loss on ignition, %	H	Heat of wetting, cal. per gm.
C	Clay, %	J	Volume expansion, %
D	Silt, %	K	Maximum moisture taken up, %
E	Moisture content at the sticky point, %	L	= B − 1.724A
F	Moisture taken up at 50% relative humidity, %	R	Multiple correlation coefficient

Equation number	Regression equation	R
1	B = 1.497A −0.00123C −0.0220D + 3.17	.936
2	E = 5.99A +0.092C +0.208D +18.2	.935
3	F = 0.356A +0.0262C −0.0119D + 1.62	.754
4	G = 1.45A +0.113C +0.123D +44.6	.710
5	H = 0.509A +0.0034C +0.0168D + 2.44	.702
6	J = 0.967A +0.143C −0.085D + 8.41	.729
7	K = 8.33A +0.170C +0.292D +28.0	.930
8	E = 3.58B +0.091C +0.261D + 9.30	.923
9	F = 0.247B +0.0267C −0.0043D + 2.28	.834
10	G = 0.982B +0.114C +0.153D +41.2	.783
11	H = 0.321B +0.00340C +0.0239D + 1.50	.728
12	J = 0.556B +0.143C −0.080D + 7.20	.708
13	K = 4.97B +0.168C +0.363D +15.7	.914
14	E = 4.10A +1.19B +0.093C +0.234D +14.7	.940
15	F = −0.356A +0.451B +0.026C −0.0020D + 0.30	.856
16	G = 3.90A −1.53B +0.114C +0.162D +47.4	.632
17	H = 0.00636A +0.318B +0.0034C +0.024D + 1.51	.728
18	J = 1.11A −0.90B +0.144C −0.087D +14.3	.725
19	K = 6.00A +1.47B +1.71C +0.325D − 9.33	.933
20	F = 0.372A +0.0266C −0.0018D +0.531L + 0.21	.868
21	G = 1.50A +0.114C +0.158D +1.86L +39.7	.791
22	F = −0.595A +0.270B +0.0147C −0.027D + 0.095E +0.0184G +0.189H −0.0167J − 0.0178K −1.39	.957
23	K = 1.63A −0.571B −0.0106C −0.071D + 1.10E −1.85F +0.67G +1.29H + 0.062J −9.30	.984

It is at first surprising to find, in regression equation No. 15 for F (moisture taken up at 50% relative humidity), that the highly significant coefficient of A (organic carbon) has a negative sign; the same is true of the highly significant coefficient of B (loss on ignition) in equation No. 16 for G (pore space). This must not be interpreted to mean that as the organic carbon content decreases, moisture taken up at 50% relative humidity will increase. Neither does it

follow that pore space decreases with an increase of loss on ignition. The opposite is, in fact, true. The explanation lies in the association which exists between the two variables, loss on ignition and organic carbon. A possible method of separating the two effects is as follows: assume that loss on ignition consists of two parts, (*a*) organic and (*b*) non-organic matter. If, as usual, the percentage of organic matter is considered to be 1.724 times as great as the percentage of organic carbon, we may calculate for each soil sample a new variable (L), the loss on ignition of material not organic, thus:

$$L = B - 1.724A,$$

which may now be treated like the other variables. When this new variable is used to replace loss on ignition in the regression equations, we find that the negative signs disappear from the equations for F and G. The resulting equations are Nos. 20 and 21 n Table VIII. This confirms the c nclusion tha the minus signs, which appear before A or B when both are used together, are due to the high association which exists between the two variables.

To examine the situation when a large number of soil properties are introduced together, the regression equations have been worked out for each of ten variables (A . . . K) in terms of the others. The results for F and K, the two which are most highly correlated with the others, are included in Table VIII to illustrate the type of result which is obtained.

Not only do these calculations supply the regression equations which express one chosen variable as a function of several others, but, following the method of Wallace and Snedecor, it is also possible to assign the weights to be attached to each of the variables and to calculate the percentage contribution of each variable to the others. Moreover, the errors in the estimates and significance of the results can be determined in all cases. This has been done, and the results are included in Table IX.

The numbers in Table IX represent the percentage contributions to the variable leading the row from the respective variables heading the columns. The third line, for example, implies that when loss on ignition is taken as the dependent variable, then the relative weights to be assigned to the independent variables organic carbon, clay, and silt are 96, 1, and 3% respectively. The first of these three values was found to have a standard error of 5.5, hence it is marked highly significant. The other two values are not significant.

The main conclusions which may be drawn from Table IX are as follows:

(i) There is very close agreement (for any particular variable) between the percentage contribution due to organic carbon or to loss on ignition, provided only one of the two variables is included in the calculation.

(ii) Organic material, measured either as organic carbon or loss on ignition, is of much greater relative importance than clay or silt. The figures indicate that, on the average, organic matter contributes between two and three times more than clay does to the dependent soil properties.

(iii) Silt shows little or no significant contribution to the other measured soil properties.

(iv) The relative contributions to moisture taken up at 50% relative humidity and to pore space from organic matter, clay, and non-organic material lost upon ignition are in the approximate ratio 7 : 3 : 4.

(v) Moisture content at the sticky point and maximum moisture taken up by the soil are dependent almost entirely upon organic material.

(vi) In no instance is clay relatively more important than organic material. This is contrary to the observations of Keen and Coutts, who found that the moisture content at 50% relative humidity was controlled largely by the clay content.

All the above observations point to the importance of the organic material in these soils in determining their physical properties. It is also evident that, as far as quantitative prediction of the dependent soil properties is concerned, organic carbon and loss on ignition are about equally useful. The latter is a much simpler determination to make. Thus, in any systematic examination of samples with a view to classification, and where time and effort must be economized, it appears that a determination of loss on ignition should be included.

TABLE IX

RELATIVE DEGREES OF ASSOCIATION BETWEEN SOIL PROPERTIES IN PERCENTAGE

–	A	B	C	D	L	E	F	G	H	J	K
F	−28**	21**	4**	−3*	—	28**	—	1	7**	1	7
K	12	7	1	1	—	53**	7	11**	7*	1*	—
B	96**	—	1	3	—						
E	85**	—	8	7	—						
F	66**	—	29**	5	—						
G	60**	—	29**	11	—						
H	68**	—	27*	5	—						
J	48**	—	43**	9	—						
K	84**	—	10*	6	—						
E	—	84**	8	8	—						
F	—	70**	28**	2	—						
G	—	61**	26**	13	—						
H	—	67**	26**	7	—						
J	—	47**	44**	9	—						
K	—	82**	10	8	—						
E	57**	28	8	7	—						
F	−28**	59**	13**	0	—						
G	52**	−34**	9**	5	—						
H	1	66	26**	7	—						
J	48	6	38**	8	—						
K	59*	24	10*	7	—						
F	49**	—	21**	1	29**						
G	46**	—	21**	10	23**						

Legend

A Organic carbon
B Loss on ignition
C Clay
D Silt
E Moisture at sticky point
F Moisture at 50% relative humidity
G Pore space
H Heat of wetting
J Volume expansion
K Maximum moisture
L = B − 1.724A

** Highly significant P = .01
* Significant P = .05

Summary

Physical properties of samples from different horizons and from different regions show the expected large range of variation.

The pipette and hydrometer methods of mechanical analysis, though generally in agreement, sometimes exhibit large discrepancies which apparently depend on the type of soil studied.

There is high correlation, both simple and partial, between many of the soil properties studied.

By means of regression equations, the dependence between the different soil properties is determined, the independent variables being sand, silt, clay, and organic matter, (or loss on ignition).

When loss on ignition is divided into (i) organic and (ii) inorganic material loss, then the relative contribution to several of the dependent soil properties from (i), (ii), and clay are in the approximate ratio 7 : 4 : 3. Thus organic material is much more important than clay in determining the physical properties of the soils studied.

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THE ACCURACY OF THE PLATE COUNT OF SUSPENSIONS OF PURE CULTURES OF BACTERIA IN STERILE SOIL[1]

By Marjorie Sutherland[2] and Norman James[3]

Abstract

A culture of *Pseudomonas fluorescens* was suspended in a sterile soil and water mixture. Dilutions of 1 : 2,000,000 and 1 : 10,000,000 were made immediately and plated in four replicates of each dilution, using nutrient agar. This was repeated 200 times. A χ^2 value was calculated from each set of four counts. The distribution of the 200 χ^2 values in the platings from each dilution agrees very well with the theoretical distribution. In a second experiment, 100 sets of four replicates of *Pseudomonas fluorescens* were plated along with 100 of *Bacterium globiforme* and 100 of a mixture of the two cultures. The distribution of the χ^2 values in each of the three sets is such that the values may be considered to have been derived from populations distributed according to the Poisson series.

The close conformity of the distribution of the actual χ^2 values to that of the expected in each of the five sets of data appears to indicate that the mean of four replicates is reliable as an estimate of the population in the dilution plated; and further, that the failure to obtain this conformity with soil flora is due to other causes than technique.

Introduction and Historical

In soil microbiology, some practical method of obtaining estimates of the individual populations in soils of various types is needed. The accurate estimation of numbers of micro-organisms may be of value in the measurement of soil potentialities or the effect of soil treatment. Present available methods are used with little knowledge of their accuracy. There is not general agreement on a test to determine how much of the variation among replicates prepared from a single sample is due to random sampling and how much is due to characters inherent in the population.

The plate method has been the most popular means of counting organisms in soil or elsewhere since Koch's development of the liquefiable solid medium in 1881. Certain limitations of the method are recognized. The result obtained does not represent a count of the actual numbers in the original sample but only of such organisms or clumps of organisms as grow to form colonies under the conditions provided. Many investigators have found numerous sources of error in the plating technique. In spite of the shortcomings of the method, a large part of the advance made in bacteriology is based

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Contribution from the Department of Bacteriology and Animal Pathology, University of Manitoba, with assistance from the National Research Council of Canada. Summary of a thesis by M. L. Sutherland submitted to the Committee on Graduate Studies, University of Manitoba, in partial fulfilment of the requirements for the M.Sc. degree.
[2] *Graduate student, University of Manitoba.*
[3] *Associate Professor of Bacteriology, University of Manitoba.*

upon plate counts. Using milk and cultures of the colon organism, Breed and Stocking (1) found that skilled analysts with proper technique usually make reasonably accurate estimates of the number of living bacteria in milk. More experience and improved practices result in more regular and supposedly more accurate counts.

One of the more recent approaches in testing the value of bacteriological data is based upon statistical methods. Fisher, Thornton and MacKenzie (4) suggested the χ^2 (Chi square) test for use on large numbers of counts of parallel plate series. The object of this test is to determine whether the variation found in a series of replicate plates is due to random sampling. Harmsen and Verweel (6) give a brief summary of the reasoning involved in the development and use of the χ^2 test.

In the test, a value of χ^2 appropriate to the Poisson distribution is worked out for each set of parallel plates by the equation

$$\chi^2 = \frac{S(x-\bar{x})^2}{\bar{x}}$$

where x is the number of colonies counted on a plate and $\bar{x}$ is the mean of the set. Fisher's table of χ^2 gives the probability of the occurrence of given χ^2 values in an infinite population for selected degrees of freedom. The P values range from .99 to .01. The class boundaries are the χ^2 values for these selected values of P. On the basis of one hundred samples, there should be one χ^2 value with a P greater than .99, one between .99 and .98, three between .98 and .95, five between .95 and .90, etc., until finally one with a χ^2 value less than .01. The χ^2 values for class boundaries are chosen for degrees of freedom equal to one less than the number of plates in the series. The observed values are placed in their respective classes and the actual compared with the theoretical or expected distribution.

A goodness-of-fit test (3) is used to ascertain the extent of this agreement. The observed and theoretical values for each class in the distribution are set up. From the formula $\frac{(actual—theoretical)^2}{theoretical}$ a value is calculated for each class. These are totalled and the probability of the occurrence of the final χ^2 value, obtained in this manner, is determined by finding the corresponding P value. A final χ^2 value with a P of .50 is accepted as indicating a perfect fit. A close agreement with the theoretical is accepted as indicating that the means of the series are reliable, and that the data give no reason for questioning the hypothesis tested. Fisher, Thornton and MacKenzie (4) believe that close agreement with the theoretical distribution is rare but possible, and that the conditions may be satisfied with simple flora or certain mixtures of organisms.

Harmsen and Verweel (6) carried out experiments with soil platings, making parallel sets of ten each. They state that their results for the total counts of bacteria and actinomycetes show too many high χ^2 values. Technique was eliminated as the cause, since their results with starch-disintegrating

organisms, protein-disintegrating organisms, and actinomycetes alone show reasonably good conformity with the theoretical distribution. Next, they grouped their results on the basis of numbers on the plates and tested the χ^2 distribution for these sets. An equally bad distribution resulted. As a last resort, this test was applied to similar data published by Waksman (12) in 1920–21, which was found to give a similar distribution when submitted to the χ^2 test. Work done in 1936 in the Bacteriology Laboratory of the University of Manitoba on a large number of field samples confirms the finding referred to above, namely, that there is some factor, other than random sampling, responsible for the variation among counts of bacteria obtained from a series of replicate platings of one dilution of field soil. A report on this and additional data from studies in 1937 will appear at an early date.

Wilson and Kullmann (13) sought to overcome this difficulty, found in plating pure cultures of rhizobia also, by discarding a χ^2 value over ten, if the variation was due chiefly to one plate. In this way they obtained a very good fit. Another method used by the same investigators was to pour a set of five plates and eliminate one, resulting in a four plate set. Any plate showing marked deviation from the other four was eliminated, otherwise, the third plate was discarded arbitrarily. It is difficult to understand how this can be done without bias.

Scope of Problem

From an examination of the literature it is evident that, before the means of replicate sets of bacterial counts may be used with confidence, it is necessary to determine the cause of the too frequent occurrence of high χ^2 values in the reports referred to above. Therefore, three points were selected for study.

1. It appeared proper to consider first whether the method of making dilutions and preparing plates, as used in this laboratory, gives reliable results when a pure culture of bacteria is used.

2. At the same time it seemed desirable to determine whether there is a change in the distribution of χ^2 values when dilutions yielding high or low counts are used.

3. The effect of mixing two pure cultures of common soil organisms was suggested as the next logical procedure.

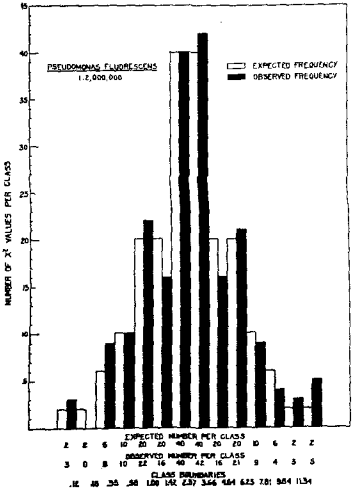
Experiment 1

This experiment was designed to examine the first and second points. It is known that non-spore-forming bacteria are more representative of the general types found in soil than the spore formers. Waksman (9) lists the common heterotrophic non-spore-formers as "*Bact. fluorescens, Bact. caudatum, Bact. radiobacter,* etc." *Pseudomonas fluorescens* was chosen as the test organism for this study. A stock culture was used. The culture was grown on agar slants for one to three days at 26° C. This time variation was used in order that the counts would not be influenced by growth phase phenomena.

In preparing the first dilution, sterile soil was added in an attempt to duplicate the effect of shaking the natural soil sample. There may be some physical or chemical condition in soil, not usually found in pure cultures from agar slants, that affects the count obtained. The dilution blanks were prepared so that there was approximately 245 ml. of water in the first and 99 ml. in the others, after sterilizing.

To prepare one set of plates, a streak culture of *Pseudomonas fluorescens* was flooded with 5 ml. of sterile water, the growth scraped from the agar and the suspension added to a 245 ml. blank, after which 25 gm. of sterile soil was introduced. This dilution was shaken for 5 min. in an automatic shaker, and dilutions were made to 1 : 2,000,000 and 1 : 10,000,000. Four plates were made from each final dilution, using one pipette for each dilution. About 10 ml. of standard nutrient agar was added to each plate.

Nutrient agar was used for plating because of its general nature. Since *Pseudomonas fluorescens* normally is not a spreading type there was little need for a medium designed to control spreaders. Bottom spreaders developed sometimes when the agar was not added immediately after the delivery of the sample to the plate. When this occurred the entire set of four plates from each dilution of the sample was discarded, as it was impossible to secure an accurate count or estimate of the original number of organisms. Contamination was considered a reason for discarding sets of plates, but excessive variation in numbers of colonies was not. The plates were incubated for three to five days, the colonies counted, and the counts checked with the hand lens. At three days, some of the colonies were missed with the unaided eye, but were found when checked with the hand lens. An increase in the number of colonies was not noted when the longer period of incubation was used, but the count was made more readily at the end of the five-day period.



HISTOGRAM 1. *χ^2 distribution on 200 samples for the 1 : 2,000,000 dilution of Pseudomonas fluorescens.*

Counts of 200 sets of four replicates of the 1 : 2,000,000 dilutions were obtained by this procedure, and another

200 sets on the 1 : 10,000,000 dilutions of the same samples. The χ^2 value for each set of counts of four replicates was calculated. These values were then distributed in their respective classes on the basis of class boundaries for three degrees of freedom, and the actual was compared with the theoretical distribution. Finally, the goodness-of-fit test, using 13 degrees of freedom, was applied to ascertain the extent of the agreement between the actual and theoretical distributions.

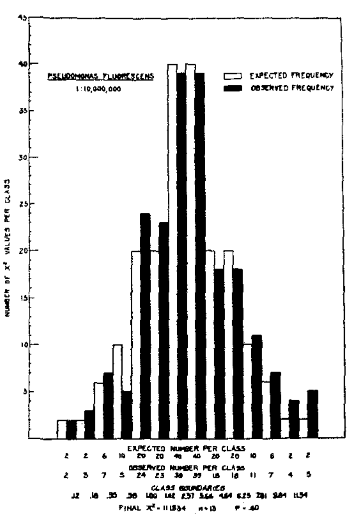
Results of Experiment 1

The actual counts, calculated means, and χ^2 values are available (7) but are not included. A summary of the actual and theoretical distributions of the 200 χ^2 values, at 1 : 2,000,000 dilution, is presented in Histogram 1, and at 1 : 10,000,000 dilution, in Histogram 2. In each case the agreement appears close. The goodness-of-fit test gives a final χ^2 value of 11.3833 and a P value of .58 at 1 : 2,000,000 dilution; and 11.1334 and .60 respectively at 1 : 10,000,000 dilution.

Experiment 2

The second experiment deals with the effect of mixing *Pseudomonas fluorescens* with a pure culture of another common soil organism. Taylor and Lochhead (8), in following up work done by Conn and Darrow (2), reported that organisms of the type of *Bacterium globiforme* represent some 10% of the organisms capable of being isolated by the plate method. This type of organism grows rapidly on nutrient agar. Dr. Lochhead very kindly provided a culture of *Bacterium globiforme* N. G. 53.

A dilution of 1 : 10,000,000 was used, since the low counts have been shown to give an equally good χ^2 distribution and involve less work. In order to have results on the separate cultures used in the mixture, sets of plates of *Pseudomonas fluorescens* and of *Bacterium globiforme* were made at the same time and from the same dilutions as used in preparing the mixtures. It was thought that

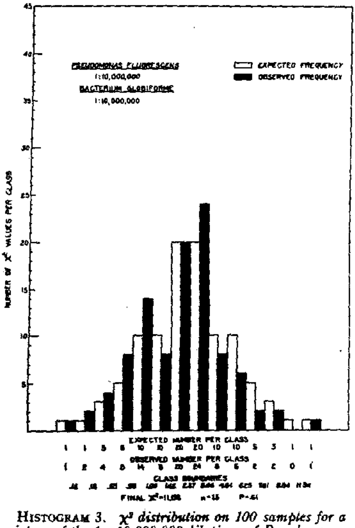


Histogram 2. *χ^2 distribution on 200 samples for the 1 : 10,000,000 dilution of Pseudomonas fluorescens.*

these might throw some light on the cause of the variation. One pipette was used for each dilution of each culture. One ml. of the final dilution of *Pseudomonas fluorescens* was placed in each of eight plates. One ml. aliquots of the final dilution of *Bacterium globiforme* were added to four of these and to four other plates. This resulted in a set of four plates each of *Pseudomonas fluorescens*, *Bacterium globiforme*, and a mixture of the two. One hundred sets of twelve plates were prepared by this procedure. The data in each set of four replicates were submitted to the mathematical treatment referred to under Experiment 1.

Results of Experiment 2

In this experiment also the actual counts and calculated values are not included; nor are the summaries of the counts on the separate sets with the pure cultures. These are available (7) and show essentially the same result as is presented in Experiment 1. The goodness-of-fit test on the distribution of the 100 samples of *Pseudomonas fluorescens* gives a final χ^2 value of 12.13 and a P value of .52; and in the 100 samples of *Bacterium globiforme* of 7.90 and ·54 respectively. In the case of the latter culture these final values were obtained by piling (3, 5) the small classes at the extremities of the histogram, thus reducing the number of classes to ten and the degrees of freedom to nine.



Histogram 3. *χ^2 distribution on 100 samples for a mixture of the 1 : 10,000,000 dilutions of Pseudomonas fluorescens and Bacterium globiforme.*

The distribution of the 100 χ^2 values, calculated from the data obtained from the plates with the two species of bacteria, is shown in Histogram 3, and represents a close agreement with what would be expected from random sampling of a population distributed according to the Poisson series. The goodness-of-fit test gives a final χ^2 value of 11.06 and a corresponding P value of .61.

Discussion of Results

The goodness-of-fit test applied to the χ^2 distribution involves an acceptance of a P value within certain definite limits. Fisher (3) states that a

range of values from .90 to .10 may be expected without questioning the hypothesis tested. A P value outside these limits may be taken as indicating that the hypothesis tested does not account for all the factors involved. In the distributions reported herein, the P values are well within and indeed are close to the mid-point of these two limits. Consequently, one may assume that the variations among counts obtained in these studies are the result of random sampling, rather than of serious error in the technique of plating and counting. The mean of four replicate plates of certain pure cultures, or mixtures of them, may be accepted as providing a reasonable estimate of the population sampled. This confirms the opinion expressed by Fisher, Thornton and MacKenzie (4), referred to in the introduction.

The finding of an equally good fit in the χ^2 distributions in the two dilutions reported under Experiment 1 is of interest from the standpoint of results obtained with field soils. The failure to obtain a good fit, when considering the total count of bacteria in the soil by the plate method, may be due to certain associative actions, antagonistic or stimulative, among organisms in the plate. The question of associative and antagonistic effects of microorganisms has been reviewed fully by Waksman (10). A high dilution means a small number of organisms in a given plate, and consequently less chance of the presence of certain antagonistic types and also less associative action because of the greater distance between organisms on the plate. However, since the variance in counts from each dilution conforms to expectation in the Poisson series, the standard error of the mean of four small-count replicates is greater than that of four large counts. In plating field soils, compensation for this loss in accuracy could be obtained by increasing either the number of plates from one dilution or the number of replicate samples plated (11).

The finding of Experiment 2 provides no indication of a disturbing factor when these two species appear in a plate. This is suggested by the χ^2 test, and by the fact that the average of the 100 sets of the mixture is 126 colonies per plate, while the sums of the separate platings average 125. Of course, this may not hold for other species of bacteria.

The results of these two studies have a definite bearing on platings from soil. Since the routine of making dilutions and plating was the same as is used with field samples of soil, the factor of laboratory technique may be eliminated as a cause of the discrepancies in the χ^2 distributions, as observed in our laboratory and reported by other investigators.

Conclusions

1. The plating technique used in this study produces sets of four replicate counts of certain pure cultures of bacteria, or mixtures of them, whose χ^2 values are distributed according to the Poisson series. The technique of diluting, plating and counting is the same as that used in this laboratory with soil samples handled on a large scale.

2. Dilutions yielding 25 to 75 colonies per plate give as good a χ^2 distribution as those from the same samples yielding five times as many colonies per plate.

3. The failure to obtain agreement between the actual and theoretical distributions of χ^2 values for counts of bacteria from soil samples is the result of factors other than the technique used in these investigations.

Acknowledgments

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EFFECT OF PHYTOHORMONES ON SEEDS DAMAGED BY FORMALDEHYDE AND OTHER DISINFECTANTS[1]

By N. H. Grace[2]

Abstract

Experiments with cereal seeds demonstrate that the reduction in germination and early growth resulting from formaldehyde treatment can be largely overcome by adding the phytohormones, 1-naphthylacetic acid or 3-indolylacetic acid, to the disinfecting solution. The optimum concentration of the hormone for individual varieties of cereals lies between 0.01 and 5 p.p.m. Similar effects were also obtained with hormones after copper sulphate and hot water treatments. The method appears to have practical possibilities, and may also be useful for comparing the physiological activities of different compounds.

Introduction

Seed treatment for the prevention of certain diseases of cereals has been practised for several centuries (10), but the advantages which result are frequently offset by the inhibiting effect of the disinfectant on germination and subsequent growth. Such effects are particularly serious with the widely used formaldehyde treatment for smut control. Seed injury occurs under most conditions and is increased when seeding is delayed after treatment, when the soil is dry, or when low-grade seed is used. As a result, this treatment is being gradually displaced by others such as those involving the use of copper carbonate and organic mercurial dust disinfectants.

Various hypotheses have been put forward to explain seed injury by disinfectants, and methods have been suggested for its reduction (1-3, 8, 9, 11). Amongst these hypotheses, that of Henry (7) seemed particularly interesting. Using the oat coleoptile as an indicator, he demonstrated that formaldehyde tends to inactivate the growth hormone, heteroauxin, and he suggested that this inactivation might account, in part, for the reduction in germination and growth caused by the disinfectant. It occurred to the writer that this hypothesis might be tested by adding a growth-promoting chemical to formaldehyde solutions used for treating seeds. If inactivation of heteroauxin is the main factor, addition of a chemical which is less sensitive to oxidation might reduce injury. Moreover, it seemed possible that such investigations might lead to the development of an improved solution method for seed treatment.

Experiments have been made with the two physiologically active chemicals, 1-napthylacetic and 3-indolylacetic acids, using a number of different types of cereal seed, and formaldehyde, copper sulphate, and hot water seed treatments.* The results demonstrate that hormones reduce the deleterious effects of the disinfectants very considerably. The method also appears to have possibilities for the comparison of the physiological activities of different chemicals.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa.
[2] *Chemist, National Research Laboratories, Ottawa.*
* *A preliminary report appeared in Nature, 142 : 77. 1938.*

Methods

Treatment

Cereal seeds were treated by sprinkling 25 gm. of seed with 5 cc. of a solution of 1 : 320 commercial formaldehyde (37% by weight of the gas) in water. This method was found to be more convenient than that of immersing the seeds in the solution for 10 min. The latter method was used in some earlier experiments, as noted later. After treatment, the samples were placed on filter paper and covered with inverted cans for 4 hr. They were then loosely wrapped in small pieces of canvas to prevent aeration and provide conditions favorable to formaldehyde damage. When planting occurred more than one day after treatment, the seed was stored in open cans to permit continued loss of moisture.

The copper sulphate treatment consisted of soaking seed for 5 min. in a solution containing 12 gm. of $CuSO_4 . 5H_2O$ in 500 cc. of water, draining, and drying for 24 hr. before planting. Hot-water disinfection involved pre-soaking the wheat for 4 hr. at 30° C., then placing the samples in solutions for 10 min. in a bath maintained at 54° C. The seed was spread in a thin layer on filter paper to cool and dry for 24 hr. before use.

Freshly prepared solutions were employed in treatment, except in a few experiments where the stability of the chemical was under test. The hormone* chemical was introduced in the treating solution and the concentration expressed in p.p.m. (parts per million) by weight.

Estimation of Effect of Treatments

Seeds were spread on wet blotting paper in 50- or 100-kernel lots and placed in a germinator at 18° C. Germination counts were made 7 to 10 days after planting.

In most of the investigations, 50 seeds were also planted in soil in small cardboard flats and kept in the greenhouse at about 16° to 20° C. After 15 to 22 days, all plants grown from the 50 seeds were collected and washed. Germination counts were made at this time. A number of plants (10, 15, 20 or 30, depending on the time and help available) were then selected at random and measured. The stem was measured from the seed to the tip of the longest leaf, and the sum of the lengths of all seminal roots was also determined. The data are recorded as mean lengths for single plants. All of the plants from 50 seeds were then dried for 4 hr. in an oven at 93° C., conditioned for one week in the laboratory, and weighed. The data are recorded as air-dry weight of plants from 50 seeds.

Experiments with Formaldehyde and Formaldehyde-hormone Treatments

Effects on Plants Grown in Soil

Data on germination, dry weights, and root and stem measurements for five pure varieties of wheat and two samples of low commercial grades are

* *For the sake of convenience, the physiologically active chemicals used in the treatments have been designated by the term "plant hormone". It is recognized that there is some question as to the accuracy of this term.*

given in Tables I and II. The low grade wheats were included since, in poor crop years, such wheats are sometimes used for seed and are particularly subject to formaldehyde damage.

TABLE I

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE-HORMONE TREATMENTS ON GERMINATION AND DRY WEIGHT OF WHEAT PLANTED IN SOIL

Treatment	Hormone concentration, p.p.m.	Germination, %						
		Varieties					Commercial wheats	
		Garnet	Marquis	Mindum	Red Fife	Reward	No. 5 wheat, 1935	No. 6 Special wheat, 1935
Untreated control		99	98	82	97	95	82	94
Formaldehyde control		74	95	63	84	65	30	82
Formaldehyde and naphthylacetic acid	0.01	74	97	80	86	94	–	92
	0.1	87	91	77	97	92	70	–
	1.0	80	96	75	94	93	78	86
	1.67	–	–	–	–	–	66	–
	3.0	75	94	83	98	88	–	80
Formaldehyde and indolylacetic acid	0.01	75	94	69	–	91	–	–
	0.1	80	94	82	–	92	74	–
	1.0	92	94	72	–	96	88	–
	1.67	–	–	–	–	–	60	–
	3.0	95	89	67	–	90	–	–
Necessary difference, 5% level		20	20	20	6	20	–	–
		Dry weight of plants from 50 seeds, gm.						
Untreated control		0.89	1.01	1.05	0.64	1.14	–	0.52
Formaldehyde-treated control		0.47	0.93	0.74	0.46	0.57	–	0.43
Formaldehyde and naphthylacetic acid treatment	0.01	0.61	1.01	1.05	0.54	1.01	–	0.46
	0.1	0.71	0.89	1.12	0.59	0.96	–	–
	1.0	0.60	0.90	0.92	0.64	0.90	–	0.43
	1.67							
	3.0	0.60	0.93	1.09	0.67	0.86	–	0.48
Formaldehyde and indolylacetic acid treatment	0.01	0.65	0.85	0.93	–	1.05	–	–
	0.1	0.64	0.94	1.10	–	1.01	–	–
	1.0	0.83	0.93	0.99	–	0.95	–	–
	1.67	–	–	–	–	–	–	–
	3.0	0.81	0.85	0.89	–	1.02	–	–
Necessary difference, 5% level		0.29	0.29	0.29	0.05	0.29	–	–

Germination data are given in the upper half of Table I as means of duplicate counts of 50 seeds. Formaldehyde damaged all samples except Marquis, and it is apparent that when damage occurred, both hormones tended to reduce it. Although the variation between duplicate counts was large, statistical

analysis shows that with Garnet, Mindum, Red Fife and Reward, a concentration of hormone was reached which resulted in a significant improvement in germination, when compared with the formaldehyde control. The commercial samples were not replicated but, though no statistical treatment is possible, the results obviously show the same trends.

TABLE II

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE-HORMONE TREATMENTS ON LENGTH OF ROOTS AND STEMS OF WHEAT PLANTS GROWN IN SOIL

Variety	Treatment	Roots, mm.	Stems, mm.
Mindum	Untreated control	392	179
	Formaldehyde control	202	170
	Formaldehyde and 0.01 p.p.m. naphthylacetic acid	488	175
	Formaldehyde and 1.0 p.p.m. indolylacetic acid	552	207
Marquis	Formaldehyde control	337	156
	Formaldehyde and 0.1 p.p.m. naphthylacetic acid	374	174
	Formaldehyde and 0.1 p.p.m. indolylacetic acid	403	163
Garnet	Formaldehyde control	158	141
	Formaldehyde and 1.0 p.p.m. naphthylacetic acid	393	176
Necessary difference, 5% level		65	10

Data on the dry weight of plants from 50 seeds are given in the lower half of Table I. The dry weight of all the pure varieties, except Marquis, is significantly reduced by formaldehyde treatment. There is a statistically significant increase above the formaldehyde control in every formaldehyde-hormone treatment of Red Fife and Reward. Garnet shows a significant increase at the one and three p.p.m. levels of indolylacetic acid, while Mindum gives the most uniform response with naphthylacetic acid. Several of the formaldehyde-hormone treatments of the four pure varieties give dry weights which are not significantly inferior to that of the untreated control. The data leave little doubt that reduction of formaldehyde damage is effected by the use of formaldehyde-hormone treatments on both high and low grades of seed.

Data on mean root and stem measurements of ten plants, selected at random, are given in Table II. Formaldehyde damage to Mindum is shown by a significant decrease in the roots. Root length is increased by the addition of 0.01 p.p.m. naphthylacetic or 1 p.p.m. indolylacetic acids, so that it becomes greater than that of the untreated control. The stem length at 1 p.p.m. indolylacetic is also significantly improved. Marquis shows a significant improvement in stems with 0.1 p.p.m. naphthylacetic acid, and a significant increase in roots with 0.1 indolylacetic acid. It is not surprising that the improvement is slight, as germination and dry weight data on Marquis indicated little damage. Garnet, however, shows a striking increase in both roots and stems as a result of the addition of hormone. The data strongly

suggest that prevention of formaldehyde damage is associated with the increased root development following the addition of hormones to the treating solutions.

Effect of Using Greater Concentrations of Hormones

Data on germination and dry weights of Red Fife wheat and Banner oats are given in Table III. The weights are for the plants grown from 50 seeds and the germination results are from both germinator and soil studies. In the range between one and five p.p.m. of naphthylacetic acid, an optimum is reached. The dry weight of wheat is significantly better than that of the untreated control at five p.p.m., and oats are better at three p.p.m. Not only has damage been completely avoided but a net stimulation occurs. A significant drop is observed at 10 and 15 p.p.m., indicating the overdosage phenomenon characteristic of plant response to these active chemicals.

TABLE III

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE PLUS HIGHER CONCENTRATIONS OF HORMONE ON GERMINATION AND DRY WEIGHT OF WHEAT AND OATS

Treatment	Hormone concentration, p.p.m.	*Germination, %*				*Dry weight of plants from 50 seeds grown in soil, gm.*	
		Germinator		Soil			
		Red Fife wheat	Banner oats	Red Fife wheat	Banner oats	Red Fife wheat	Banner oats
Untreated control		95	93	97	99	0.64	0.93
Formaldehyde control		83	91*	84	78	0.46	0.62
Formaldehyde and naphthylacetic acid	0.01	95		86		0.54	
	0.1	98	95	97	93	0.59	0.83
	1	98	98	94	94	0.64	0.82
	3	98	93	98	100	0.67	1.10
	5	99	93	92	87	0.69	0.83
	10	95	97	93	84	0.59	0.78
	15	89		92		0.55	
Necessary difference, 5% level		6	4	6	15	0.05	0.16

* *The wheat was treated with the usual 1 : 320 formaldehyde solution, but the oats with 1 : 180, as the higher concentration was required to show damage.*

Comparison of Commercial Formaldehyde and Polymer-free Formaldehyde

It has been suggested that formaldehyde damage is associated with a layer of paraformaldehyde which forms on the surface of the treated seed during drying. Earlier work in this laboratory showed that polymer-free formaldehyde caused essentially the same damage as the commercial product which contains some polymer, and that substantial increase in the methyl alcohol content had little effect. While it appeared unlikely that the presence of polymer would seriously affect the extent of damage, this aspect has received consideration. A sample of polymer-free formaldehyde* prepared in an

* *Polymer-free formaldehyde specially prepared by the Standard Chemical Company, Montreal.*

excess of methyl alcohol was compared with a commercial sample which had been exposed to low temperature in order to increase the amount of polymer. It was also of interest to ascertain the effect of variation in polymer content on the response to formaldehyde-hormone treatments.

The data on germination and dry weight of plants grown in soil (Table IV) are for two low commercial grades of wheat. They indicate that there is little difference in the response to these two different formaldehyde solutions. While the treatments in soil were not replicated, and consequently the data cannot be tested for significance, it is evident from the dry weights that somewhat greater damage to growth may be caused by the polymer-free formaldehyde. Improvement in germination and early growth from the use of formaldehyde-hormone solutions appears to be independent of the content of polymer.

TABLE IV

EFFECT OF COMMERCIAL AND POLYMER-FREE FORMALDEHYDE ON GERMINATION AND DRY WEIGHT OF COMMERCIAL WHEATS

Treatment	Hormone concentration, p.p.m.	Germination, %				Dry weight of plants from 50 seeds grown in soil, gm.	
		Germinator		Soil			
		No. 5 wheat, 1935	No. 6 Special wheat, 1935	No. 5 wheat, 1935	No. 6 Special wheat, 1935	No. 5 wheat, 1935	No. 6 Special wheat, 1935
Untreated control		84	84	90	94	0.72	0.52
Commercial formaldehyde control		67	68	86	82	0.52	0.43
Polymer-free formaldehyde control		72	65	82	82	0.40	0.38
Commercial formaldehyde and hormone*	0.01	76	82	86	92	0.71	0.46
	1.0	72	71	90	86	0.67	0.43
	3.0	78	79	88	80	0.66	0.48
Polymer-free formaldehyde and hormone*	0.01	74	91	88	90	0.65	0.46
	1.0	81	80	88	82	0.70	0.39
	3.0	82	86	88	88	0.57	0.53
Necessary difference, 5% level		8	8	—	—	—	—

* *No. 5 wheat treated with formaldehyde-indolylacetic acid; No. 6 special wheat treated with formaldehyde-naphthylacetic acid.*

Effects of Time Interval between Treatment and Seeding

It has been pointed out that formaldehyde treatment usually causes some injury to the seed, and that this is increased when seeding is delayed or the soil is dry. In consequence, the response of formaldehyde-hormone-treated seed to delayed planting, or planting in dry soil, is of interest.

Data are given in Table V for the germination of No. 1 Northern wheat treated by immersion. This experiment was one of the earlier ones. Others which it seems unnecessary to report showed that immersion and sprinkling

gave essentially similar results, and in later work the more convenient sprinkling method was used.

The data show that germination of the formaldehyde control is reduced when seed is planted one day after treatment, and that germination is further markedly reduced when the time between treatment and planting is extended to two and eight days. These effects are, however, substantially offset in all the formaldehyde-hormone treatments. A somewhat better response is obtained with naphthylacetic acid, particularly with seed planted eight days after treatment. However, individual blotting-paper flats of 100 seeds were used and the data cannot be tested for significance.

TABLE V

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE-HORMONE TREATMENTS ON PERCENTAGE GERMINATION OF NO. 1 NORTHERN WHEAT PLANTED ONE, TWO AND EIGHT DAYS AFTER TREATMENT*

Treatment	Hormone concentration, p.p.m.	Germination, %		
		1 day	2 days	8 days
Untreated		82	—	—
Soaked in water only		74	74	66
Formaldehyde control		58	0	4
Formaldehyde and naphthylacetic acid	0.1	76	78	62
	1.0	82	86	66
	5.0	78	68	66
Formaldehyde and indolylacetic acid	0.01	68	78	52
	0.10	80	60	56
	1.0	72	58	50

* *Treatment by immersing the seed in all cases. Seed planted in germinator.*

Data are given in Table VI for the germination of Laurel (hull-less) oats on blotting paper and in wet and dry soil. The solutions used in these treatments were prepared from stock formaldehyde-hormone solutions which had been held in glass containers for 23 days. The germinator results showed improved germination for seed planted one and two days after treatment, and the improvement is somewhat more marked for the germination of seed planted in soil. Seed planted in dry soil was watered two days after planting. It is apparent that the two-day period in dry soil has not increased damage, but a longer period of exposure to this condition might have done so.

Improvement in germination over the formaldehyde control thus results from formaldehyde-hormone treatment even if planting is delayed for several days after treatment.

Effect of Storing Stock Solutions of Hormone Chemicals in Commercial Formaldehyde

It has been shown that freshly prepared formaldehyde-hormone solutions can be used to advantage in the treatment of seed. Since the practical

application of hormone chemicals for this purpose requires their addition to commercial formaldehyde by the manufacturer, and since several weeks usually intervene between packaging and use, stability of the chemical is of major importance. A series of experiments was carried out to determine the effect of storage period on the physiological activity of hormones dissolved in commercial formaldehyde.

TABLE VI

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE-HORMONE TREATMENTS ON PERCENTAGE GERMINATION OF LAUREL (HULL-LESS) OATS PLANTED ONE AND TWO DAYS AFTER TREATMENT*

Treatment	Hormone concentration, p.p.m.	Germination, %					
		Germinator		Soil			
				Moist		Dry†	
		1 day	2 days	1 day	2 days	1 day	2 days
Untreated		96	90	92	98	88	98
Formaldehyde control		67	54	48	52	46	56
Formaldehyde and naphthylacetic acid	0.1	82	72	72	66	66	76
	1.0	78	84	88	80	72	84
Formaldehyde and indolylacetic acid	0.1	70	76	66	76	74	72
	1.0	78	84	54	68	84	76
Necessary difference, 5% level		9	–	–	–	–	–

* *The stock formaldehyde-hormone solutions had been held for 23 days in glass containers.*
† *The samples in dry soil were watered two days after planting.*

Hormone chemicals were added to formaldehyde solution containing 37% of the gas by weight, to give 3.2, 32 and 320 p.p.m. in the stock solutions. Subsequent dilution of 1 : 320 gave treating solutions containing 0.01, 0.1 and 1 p.p.m. of hormone.

The data in Table VII give mean root and stem measurements on 20 plants of wheat and oats selected at random from each lot, and compare the effect of seed treatment with fresh formaldehyde-hormone solutions and solutions from stock preparations held in glass containers for 10 weeks. Both wheat and oats show significant root damage from formaldehyde alone and this damage is significantly reduced throughout by the use of formaldehyde-hormone treatments. Improvement in oat stems is found with the new solutions, while the stem differences of wheat are not significant. It is evident that the physiological activity of naphthylacetic acid has been maintained for the period of 10 weeks, and that the differences between old and new solutions are not statistically significant. Since essentially similar results are obtained with stock solutions of indolylacetic acid, there is reason to think that the stability of the hormone chemical in commercial formaldehyde will be satisfactory for practical application in seed treatment.

Some indication of this was already afforded by the data in Table VI on the germination of hull-less oats treated with solutions made from stock preparations held in glass for 23 days. Since formaldehyde-hormone treatments with fresh solutions are not included, a direct comparison is impossible. However, the increase in germination from hormone treatment indicates that physiological activity is still in evidence after holding either indolylacetic or naphthylacetic acids in commercial formaldehyde for 23 days.

TABLE VII

EFFECT ON PHYSIOLOGICAL ACTIVITY OF STORING STOCK SOLUTIONS OF NAPHTHYLACETIC ACID IN COMMERCIAL FORMALDEHYDE FOR TEN WEEKS IN GLASS CONTAINERS

Solution	Hormone concentration, p.p.m.	No. 3 Northern wheat		Laurel (hull-less) oats	
		Root length, mm.	Stem length, mm.	Root length, mm.	Stem length, mm.
Untreated control		373	181	249	156
Formaldehyde control		291	164	154	145
Formaldehyde and naphthylacetic acid					
Stored 10 weeks	0.01	396	176	199	153
Fresh	0.01	350	164	229	161
Stored 10 weeks	1.0	370	174	231	156
Fresh	1.0	380	176	212	161
Necessary difference, 5% level		54	15	42	12

Effect of Hormone Application after Seed is Treated with Formaldehyde and Dried

It would seem that hormone chemicals in formaldehyde solutions either reduce initial seed injury or provide some essential factor which subsequently enables the plant partially to overcome existing damage. Stoichiometrical considerations make it unlikely that the hormone directly affects the activity of the formaldehyde itself. In the following experiments, two different samples of wheat were treated with hormones one day after formaldehyde treatment and drying. In the first experiment, treated seed was washed in water and a portion was washed in 0.01 p.p.m. naphthylacetic acid. The seed was planted seven days after this treatment, and the germination percentages were: formaldehyde control, 4%; washed in water, 44%; washed in 0.01 p.p.m. hormone solution, 56%.

In the second experiment, the seed was dusted with talc at ½ oz. per bushel, giving talc and talc-hormone treatments* of 10 and 50 p.p.m. of indolylacetic and phenylacetic acids. The seed was planted immediately and gave germination percentages of 38, 66 and 58 respectively. The resulting stimulation of early growth was as marked as the effect on germination. In consequence, it seems probable that the effect of hormone on formaldehyde-treated seed should be attributed to subsequent stimulation of damaged seed rather than to prevention of damage at the time of treatment.

* *Dust treatments refer to parts by weight of hormone chemical applied to a million parts of seed. Solution treatments, of necessity, merely indicate the hormone concentration.*

Experiments with Hormones in Seed Disinfection by Means of Copper Sulphate or Hot Water

Copper Sulphate Treatment

Data on germination and dry weight of plants from wheat treated with copper sulphate and copper sulphate-hormone mixtures are given in Table VIII. It is apparent that hormone has little effect on germination, though stimulation is suggested at three p.p.m. with No. 5 special wheat. An increase occurs in the dry weight of plants from 50 seeds, except with No. 5 special wheat at one p.p.m. of naphthylbutyric* acid.

TABLE VIII

EFFECT OF COPPER SULPHATE AND COPPER SULPHATE-HORMONE TREATMENTS ON GERMINATION AND DRY WEIGHT OF WHEAT PLANTED IN SOIL

Treatment	Hormone concentration, p.p.m.	Germination, %		Dry weight of plants from 50 seeds, gm.	
		No. 5 Special wheat, 1935	Feed wheat, 1935	No. 5 Special wheat, 1935	Feed wheat, 1935
Untreated control		92	92	0.56	0.64
Copper sulphate control		62	66	0.36	0.33
Copper sulphate and naphthylacetic acid	0.1	54	58	0.41	0.43
	1.0	62	58	0.40	0.43
	3.0	86	52	0.58	0.39
Copper sulphate and naphthylbutyric acid	1.0	52	52	0.33	0.47

Root and stem measurements are given in Table IX as means of 15 plants chosen at random from each treatment of feed wheat. Seed injury by copper sulphate is shown by significant reduction in the length of roots, and copper sulphate-hormone treatment increases roots significantly above the mean value for the copper sulphate control. The result at one p.p.m. is an exception, as the increase is not significant. It is evident that copper sulphate treatment has not reduced the stems of feed wheat, and with added hormone

TABLE IX

EFFECT OF COPPER SULPHATE AND COPPER SULPHATE-HORMONE TREATMENTS ON THE LENGTH OF ROOTS AND STEMS OF FEED WHEAT PLANTED IN SOIL

Treatment	Roots, mm.	Stems, m
Untreated control	306	207
Copper sulphate control	226	207
Copper sulphate and 0.1 p.p.m. naphthylacetic acid	275	231
Copper sulphate and 1.0 p.p.m. naphthylacetic acid	257	237
Copper sulphate and 3.0 p.p.m. naphthylacetic acid	272	219
Copper sulphate and 1.0 p.p.m. naphthylbutyric acid	305	240
Necessary difference, 5% level	41	18

* *The naphthylbutyric acid used in all these experiments is a mixture of 1- and 2-γ-naphthylbutyric acids.*

the stems of the treated samples are, with an exception at 3 p.p.m., all significantly longer than those of the untreated control.

Root and stem measurements also were made on 20 plants from the copper sulphate control and 3 p.p.m. naphthylacetic acid groups of No. 5 special wheat. The average roots were 226 and 314 mm. respectively, an actual difference of 88 with 55 mm. required for the 5% level of significance. Similarly, the stem increase of 27 mm. is significant.

Hot Water Treatment

In Table X are given the percentage germination in the germinator and in soil of three different wheats treated with hot water and hot solutions of naphthylacetic acid. The data for growth in soil show that the hormone treatment has increased the rate of germination with each sample of wheat, though there is no difference in the total germination of feed wheat. The germination on blotting paper is improved with feed and Huron wheats, but not with No. 5. While the blotting paper germination of No. 5 wheat fails to show improvement from hormone treatment, there was substantially better growth.

TABLE X

EFFECT OF HOT WATER AND HOT HORMONE SOLUTION TREATMENTS ON GERMINATION OF WHEAT

Variety or grade of wheat	Treatment	Germination, %					
		Germinator	Soil				
			Days after planting				
			5	6	7	8	20
Feed, 1935	Untreated control	72	72	84	86	88	88
	Hot water control	58	2	12	30	34	68
	Hot naphthylacetic acid solution, 1 p.p.m.	72	4	32	48	56	64
	Hot naphthylacetic acid solution, 10 p.p.m.	62	0	2	38	56	68
No. 5, 1935	Untreated control	73	66	86	86	86	86
	Hot water control	52	0	4	8	8	22
	Hot naphthylacetic acid solution, 1 p.p.m.	34	0	0	4	14	24
	Hot naphthylacetic acid solution, 10 p.p.m.	31	0	0	8	28	40
Huron	Untreated control	56	52	56	60	66	66
	Hot water control	10	0	4	4	4	22
	Hot naphthylacetic acid solution, 1 p.p.m.	36	4	16	28	34	40
	Hot naphthylacetic acid solution, 10 p.p.m.	36	28	50	58	58	64

Data are given in Table XI on root and stem measurements as means of 10 plants selected at random from each treatment. Hot water treatment of Huron and No. 5 wheat results in a significant reduction in the total length of roots and stems. Treatment in hot hormone solution increases the roots of No. 5 wheat significantly, but has little effect on the length of stem. There is a striking increase in the stems of Huron following hormone treatment. The increase in roots at one p.p.m. is not significant, while the 10 p.p.m. treatment gives roots significantly longer than those of the untreated control.

While the absence of damage to feed wheat by the hot water treatment cannot be explained, the lack of marked hormone stimulation is, as already indicated, the expected result in such circumstances.

TABLE XI

EFFECT OF TREATMENT WITH HOT WATER AND HOT HORMONE SOLUTION ON LENGTH OF ROOTS AND STEMS OF WHEAT PLANTED IN SOIL

Treatment	No. 5 wheat, 1935		Huron		Feed wheat, 1935	
	Roots, mm.	Stems, mm.	Roots, mm.	Stems, mm.	Roots, mm.	Stems, mm.
Untreated control	238	220	348	344	226	177
Hot water control	163	191	262	241	240	192
1 p.p.m. naphthylacetic acid	208	192	276	324	207	207
10 p.p.m. naphthylacetic acid	233	198	432	341	223	188
Necessary difference, 5% level	37	28	56	36	—	—

Determination of Physiological Activity by the Response of Formaldehyde-damaged Seed

The response of damaged seed may be used as a method of determining the physiological activity of chemicals (6). It is essential that seed susceptible to formaldehyde damage be used. The subsequent improvement effected by the test solution then gives a measure of its activity. The method is advantageous because of its simplicity and gives results which are in close agreement with those obtained by other methods.

A number of pure chemicals were tested for physiological activity by the response of low grades of wheat from the 1935 crop. Data on germination and dry weight of plants grown from 50 seeds in soil are given for a series of isolated experiments in Table XII. Duplicates of 50 seeds were germinated on blotting paper and the necessary difference for the 5% level of significance is given. Since the data for germination and dry weight of plants grown in soil are for individual flats, they cannot be examined statistically. Nevertheless, they serve to confirm the results obtained with the germinator. It will be observed that formaldehyde damage is significant throughout. With No. 5 special wheat (A), the greatest reduction in damage is effected by naphthylbutyric acid, phenylacetic acid (which has already been recognized as having activity) giving somewhat less protection against damage. Vanillin and methoxysalicylaldehyde are intermediate in effect, with benzoic acid and piperonal showing activity of a lower order. The improvement caused by coumarin, sulphanilamide (para-amino benzene sulphonamide), and Vitamin B_1 indicates physiological activity. Pyrrole acetic acid appeared to have no effect.

Data are given at the bottom of Table XII on the response to treatment with a mixture of one p.p.m. each of ethyl mercury bromide and naphthylacetic acid. While the use of organic mercurial dust disinfectants as carriers for

hormone chemicals has been reported (5), it is of some interest to determine the effect when formaldehyde solutions are fortified with a second disinfectant. The results with No. 4 wheat indicate that the addition of organic mercury does not restrict the activity of the hormone.

Data on germination, dry weights, and measurements on the roots and stems of wheat grown in soil are given in Table XIII. The germination and

TABLE XII

PHYSIOLOGICAL ACTIVITY OF VARIOUS CHEMICALS AS INDICATED BY THE RESPONSE OF FORMALDEHYDE-DAMAGED SEED

Variety or grade of wheat	Treatment*	Germination, %		Weight of plants, gm.
		Germinator	Soil	
No. 5 Special wheat (A), 1935	Untreated control	87	92	0.87
	Formaldehyde control	6	2	0.002
	Formaldehyde and naphthylbutyric acid	80	68	0.67
	Formaldehyde and phenylacetic acid	71	54	0.59
	Formaldehyde and piperonal acid	68	32	0.32
	Formaldehyde and benzoic acid	73	48	0.36
	Formaldehyde and methoxysalicylaldehyde	68	44	0.57
	Formaldehyde and vanillin	69	48	0.52
	Necessary difference, 5% level	9	—	—
No. 5 wheat, 1935	Untreated control	85	88	
	Formaldehyde control	0	0	
	Formaldehyde and coumarin	66	34	
	Formaldehyde and sulphanilamide	69	70	
	Necessary difference, 5% level	6	—	
No. 5 Special wheat (B) 1935	Untreated control	88	92	0.56
	Formaldehyde control	72	70	0.37
	Formaldehyde and colchicine	80	72	0.46
	Necessary difference, 5% level	5	—	—
Feed wheat, 1935	Untreated control	77	80	0.43
	Formaldehyde control	56	70	0.30
	Formaldehyde and Vitamin B_1	76	82	0.39
	Necessary difference, 5% level	10	—	—
No. 3 Northern wheat, 1935	Untreated control	90		
	Formaldehyde control	68		
	Formaldehyde and 0.1 p.p.m. indolylacetic acid	88		
	Formaldehyde and pyrroleacetic acid	66		
	Necessary difference, 5% level	11		
No. 4 Northern wheat, 1935	Untreated control	80	86	0.58
	Formaldehyde control	58	66	0.45
	Formaldehyde and ethyl mercury bromide and naphthylacetic acid	80	78	0.58
	Necessary difference, 5% level	8	—	—

* *1 p.p.m. of the chemical under test was added to the formaldehyde solution.*

TABLE XIII

EFFECT OF COLCHICINE AND VITAMIN B_1 ON GERMINATION, DRY WEIGHT, AND ROOT AND STEM MEASUREMENTS OF FORMALDEHYDE-DAMAGED WHEAT PLANTED IN SOIL

Variety or grade of wheat	Treatment	Germination, %	Dry weight of plants from 50 seeds, gm.	Root length, mm.	Stem length, mm.
Feed, 1935	Untreated control	92	0.64	306	207
	Formaldehyde control	66	0.36	196	204
	Formaldehyde containing 1 p.p.m. colchicine	86	0.58	266	223
	Necessary difference, 5% level	—	—	41	18
Huron	Untreated control	82	1.04	399	243
	Formaldehyde control	54	0.46	261	216
	Formaldehyde containing 1 p.p.m. Vitamin B_1	72	0.63	317	236
	Necessary difference, 5% level	—	—	63	22

dry weight data are essentially similar to those of Table XII. There is a statistically significant decrease in roots from formaldehyde treatment of both feed and Huron wheat. While colchicine effects a significant increase in both roots and stems of feed wheat, the improvement effected by Vitamin B_1 on Huron wheat is just below the level of significance.

Effect of Hormone Solutions on Untreated Seed

A number of control experiments were made in order to test the effect of hormone solutions on untreated seed. The data are summarized in Table XIV, which gives percentage germination and dry weights, and some green weights, of plants grown from three commercial grades of wheat treated with solutions of hormone. There is no significant difference in germination of No. 5 wheat, but an increase at 0.01 and 3 p.p.m. with No. 6 special. The dry weights of plants of these two samples, grown in soil, fail to suggest appreciable increase from hormone treatment. It is apparent that germination and green weights of No. 1 Northern wheat show a significant amount of damage from hormone treatment, the damage reaching a peak at 50 p.p.m. These observations indicate that the response of damaged seed to treatment with solutions of hormones must be associated with the damaged condition, rather than to any normal growth stimulation.

It is also interesting to note that when seed is treated with hormone or formaldehyde-hormone solutions and dried previous to germination, a reduced growth of common molds is frequently observed. This phenomenon has not yet been investigated quantitatively. However, as the effect of these solutions on the growth of molds is a matter of some importance, the results of our preliminary qualitative investigation are reported in order to illustrate the sterilizing effect of naphthylacetic acid.

Duplicate malt agar plates of (A) untreated wheat, (B) wheat surface sterilized by soaking for 15 minutes in calcium hypochlorite solution (2%

TABLE XIV

EFFECT OF SOLUTIONS OF HORMONE ALONE ON GERMINATION AND WEIGHT OF WHEAT

Treatment	Hormone concentration, p.p.m.	Germination, %					Weight of plants from 50 seeds grown in soil		
		Germinator		Soil			Dry weight, gm.		Green weight, gm.
		No. 5 wheat, 1935†	No. 6 Special wheat, 1935*	No. 1 Northern wheat*	No. 5 wheat, 1935†	No. 6 Special wheat, 1935*	No. 5 wheat, 1935†	No. 6 Special wheat, 1935*	No. 1 Northern wheat*
Untreated control		84	84	98	90	94	0.72	0.52	12.6
Hormone solutions	0.01	85	93		96	94	0.77	0.54	
	1.0	76	88		96	92	0.71	0.51	
	3.0	88	93		96	96	0.67	0.46	
	5.0			91					12.5
	10.0			92					12.4
	20.0			89					12.3
	50.0			77					9.6
	100.0	80		90	90		0.69		11.6
Necessary difference, 5% level		8	8	7					1.3

* *No. 1 Northern wheat and No. 6 Special wheat treated with naphthylacetic acid.*

† *No. 5 wheat treated with indolylacetic acid.*

available chlorine), and (C) wheat soaked 12 hr. at 32° C. in a 100 p.p.m. solution of naphthylacetic acid, are shown in Plate I. It is apparent that the hormone treatment reduces the growth of molds without reducing the development of bacterial colonies. Although general conclusions cannot be drawn from the results of this and other similar experiments made so far, the evidence suggests that further investigation might be profitable.

General Discussion

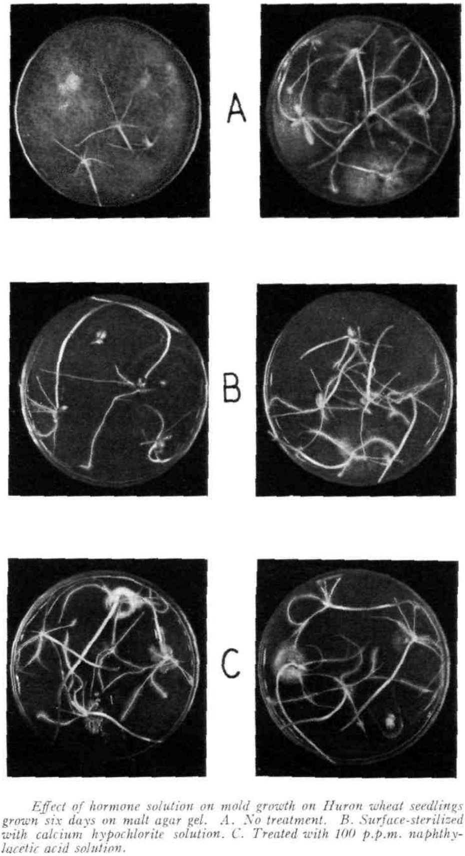
Seed injury from disinfection with formaldehyde causes reduced root growth, and generally, some reduction in the length of stem of 2- to 3-weeks-old wheat and oat plants. Increased root development is the most marked response after addition of physiologically active chemicals to the disinfectant solution. There is also a tendency to increase the stem length. These observations would appear to substantiate Henry's suggestion that formaldehyde damage is due to the inactivation of the seed's natural growth hormone. The large number of substances capable of reducing the injury, and the absence of inactivation when indolylacetic acid is added in the formaldehyde solution, suggest that the response is not so simple. It is possible that a precursor, or accessory factor, of the normal growth-promoting substance is affected by the formaldehyde. The mechanism may involve the enzyme system.

A recent paper by Clark (4) suggests that functional materials such as hormones, vitamins, bios, auxins and growth regulators manifest themselves through the activation of some enzyme. Clark's suggested mechanism for the action of these substances fits in with the observations of Atwood (1), who found that the diastatic activity of formaldehyde-treated grain is retarded. Whether injury is a direct effect on the auxins, or is related to some accessory factor, or to changes in the enzyme system, the addition of hormone chemicals permits readjustment and thereby reduces damage. It is apparent that the application of physiologically active chemicals to reduction of seed injury should be helpful in elucidating the mechanism involved.

The practical application of hormones in seed disinfection offers interesting possibilities. While laboratory observations have been limited to the effect on germination and early growth, field experiments must determine the effect on incidence of smut and final yield. It is evident that much of the objection to the formaldehyde treatment has been removed if results in the field are essentially similar to those in the laboratory. It will be possible for the manufacturer to add the required amount of chemical to commercial formaldehyde at the time of final packing, since the chemicals can be held in this way for at least 10 weeks without loss in activity. This procedure should eliminate the hazard of damage from overdosage and render the use of hormones in seed treatment exceedingly simple.

There are similar possibilities in the use of hormone chemicals in the hot water treatment for loose smut. As this method must be used when seed is infected with smut, and serious seed injury may result, the use of hormones should increase the margin of safety as well as improve the growth from the seed which does germinate.

PLATE I



Effect of hormone solution on mold growth on Huron wheat seedlings grown six days on malt agar gel. A. No treatment. B. Surface-sterilized with calcium hypochlorite solution. C. Treated with 100 p.p.m. naphthylacetic acid solution.

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INACTIVATION OF SEED-BORNE PLANT PATHOGENS IN THE SOIL[1]

BY A. W. HENRY[2] AND J. A. CAMPBELL[3]

Abstract

Certain seed-borne pathogens are inactivated to a marked degree when infested seed is sown in natural soil. *Polyspora lini* and *Colletotrichum lini*, the fungi causing respectively the Browning and Anthracnose diseases of flax, are so affected, both when naturally and artificially infested seed is used. This appears to be due largely to the antibiotic action of the micro-organisms of the soil, since in sterilized soil similar seed produces significantly higher percentages of infection. Infection may be reduced as much or more by this means as by seed treatment with certain fungicides.

On the contrary, some seed-borne pathogens apparently are not inactivated to such an extent as to produce consistently less disease in natural than in sterilized soil. This has been indicated by preliminary experiments with certain smut fungi, for example those causing bunt of wheat.

Introduction

It has become recognized in recent years that much potential inoculum of plant pathogenic fungi and bacteria, which finds its way into the soil with the residues of diseased plants, is rendered innocuous owing to the antibiotic action of normal soil micro-organisms, and in consequence does not function in the initiation of disease.

The question arises as to the fate of pathogens which are deposited in the soil on or in infested seeds. It is well known that many plant pathogens regularly enter the soil in this manner. While seed-borne inoculum is obviously in an advantageous position for infection so far as proximity to the developing seedling is concerned, that which is carried externally must become exposed to the influence of other micro-organisms in the soil as soon as the seed is sown. Inoculum present in the interior of seeds obviously would be less subject to such action, but after rupture of the seed coats it is possible that even some of it might be affected.

It seemed that biological antagonism in the soil might explain a number of cases both in the field and greenhouse, where we have observed heavily infested seed to yield lightly diseased crops. It has been customary in the past to explain such occurrences on the basis of unfavorable temperatures or other physical factors. The object of these studies was to determine the possible

[1] *Received July 2, 1938.*
Contribution from the Department of Field Crops, University of Alberta, Edmonton, Alberta.
[2] *Associate Professor of Plant Pathology, University of Alberta, Edmonton.*
[3] *Assistant in the Division of Forage Crops, Dominion Department of Agriculture, Swift Current, Sask., formerly graduate student in Plant Pathology, University of Alberta.*

importance of the biological factor, that is, the antagonistic action of soil micro-organisms to certain seed-borne pathogens. The pathogens chosen were those causing the Browning and Anthracnose diseases of flax, namely, *Polyspora lini* Laff. and *Colletotrichum lini* (West.) Toch., and the fungi causing covered smuts of wheat, oats and barley, namely, *Tilletia* spp. *Ustilago levis* (K. & S.) Mag., and *U. hordei* (Pers.) K. & S.

Literature Review

Christensen (1) investigated the effect of the soil microflora on the severity of seedling blight of barley caused by *Helminthosporium* and *Fusarium*. When naturally infected seed was used, no reduction in severity of seedling injury occurred in non-sterilized soil as compared with sterilized soil. Moreover, the addition of *Trichoderma lignorum* and several other fungi and bacteria or their extracts to naturally infected seed did not lessen the severity of the seedling blight. However, these organisms, when added to seed or sterilized soil artificially infested with *Helminthosporium sativum*, did suppress the pathogen as evidenced by decreased seedling damage.

Novogrudski *et al.* (2) studied the effect of treating diseased flax seed with certain soil-inhabiting bacteria on the severity of disease in the resulting seedlings. *Bacillus fluorescens* and *B. mesentericus* reduced the number of diseased seedlings when added to diseased seed of one variety of flax, but had no appreciable effect on another. *B. megaterium* reduced disease in both varieties, while *B. mycoides* as a rule increased it.

Experiments with Flax Pathogens

Having noticed instances where samples of flax seed, heavily infested with *Polyspora lini* Laff. or *Colletotrichum lini* (West.) Toch., failed to produce severely diseased stands of seedlings when sown in natural soil, it was decided to select these fungi for study. Both are seed-borne pathogens, and both attack the seedlings at an early stage. The former causes the Browning disease and the latter, Anthracnose. Cotyledonary lesions are commonly produced by both organisms so that diseased plants can be detected at an early stage in their development, in fact very soon after they emerge.

On the hypothesis that low infection of seedlings produced from heavily inoculated seed might be due to the inactivation of the seed-borne pathogens by the mirco-organisms of the soil, plantings of infested seed were made in sterilized and natural soil. After the seedlings emerged, comparative records were made of the severity of cotyledonary infection of plants in the two types of soil. Steam at 15 lb. pressure was used to sterilize the soil. During and following seeding, the sterilized soil was exposed to contamination, though in the first experiment the sterilized pots were kept covered with sterilized paper until the seedlings were well emerged.

Results with *Polyspora lini*

For the studies with *Polyspora lini* a sample of Bison seed flax was used. This had been obtained from a farmer who had observed disease damage in

his crop. *Polyspora lini*, the fungus causing the Browning disease, was readily isolated from a high percentage of the seeds of this sample, showing that it was naturally infested. Moreover, the variety Bison is known to be highly susceptible to this fungus, and hence was especially suitable.

In one experiment a portion of this Bison seed was divided into two parts, one of which was sown as it was, while the other was first artificially inoculated by dipping it into a suspension of spores from a culture of *Polyspora lini*. The two samples of seed were then sown in 6-in. pots at the rate of 50 seeds per pot. The soil used consisted of three parts of black soil to one part of sand. Half of the pots of soil were sterilized. The pots were kept covered with paper until after emergence had taken place. The amount of disease was noted 16 days after seeding. The total number of seedlings per pot was counted, and the number showing definite cotyledonary lesions typical of those produced by *P. lini*. The results of this experiment are recorded in Table I.

TABLE I

COMPARATIVE SEVERITY OF THE BROWNING DISEASE OF BISON FLAX CAUSED BY *Polyspora lini* IN STERILIZED AND NATURAL SOIL

Infestation of seed	Sterilized soil			Natural soil		
	Number of seedlings		Percentage diseased	Number of seedlings		Percentage diseased
	Diseased	Healthy		Diseased	Healthy	
Naturally	4	35	10.3	1	33	2.9
	8	35	18.6	1	35	2.8
	5	31	13.9	1	38	2.6
	2	36	5.3	0	39	0
			Av. 12.0			Av. 2.1
Naturally and artificially	9	30	23.1	1	36	2.7
	8	35	18.6	3	36	7.7
	6	38	13.6	1	34	2.9
	7	36	16.3	3	33	8.3
			Av. 17.9			Av. 5.4

A consistent and marked reduction in the amount of seedling infection is evident in the natural soil as compared with the sterilized soil.* It is of interest to note that this is true in the series from naturally infested seed as well as in that from naturally plus artificially infested seed. The difference in each case between the number of diseased seedlings in sterilized and natural soil is definitely significant, as judged by a simple Chi-square test. The values of χ^2 obtained, 10.19 for the naturally infested seed and 10.12 for the naturally and artificially infested seeds, are well above the 1% point, so that one may safely conclude that the disease in both cases is more severe in the sterilized soil.

* *Method of analysis kindly suggested by Dr. C. H. Goulden.*

In a second experiment a portion of the same sample of Bison seed was used, and the same procedure followed but with the addition of two series in which treated seed was sown. The treatments, which were applied to naturally infested seed only, were used in order to destroy inoculum on the surface. One treatment consisted of dipping the seed in 95% ethyl alcohol and burning off the alcohol in a flame, the other consisted of dusting it thoroughly with methyl mercury phosphate. All seed samples were then sown in both sterilized and natural soil. The results of this experiment are given in Table II.

TABLE II

COMPARATIVE SEVERITY OF THE BROWNIHG DISEASE OF BISON FLAX IN SEEDLINGS FROM TREATED AND UNTREATED SEED IN STERILIZED AND NATURAL SOIL

Seed treatment	Infestation of seed	Sterilized seed			Natural soil		
		Number of seedlings		Percentage diseased	Number of seedlings		Percentage diseased
		Diseased	Healthy		Diseased	Healthy	
None	Natural and artificial	37	90	29.1	4	92	4.2
None	Natural	31	134	18.8	2	106	1.9
Alcohol (flamed)	Natural	10	149	6.3	0	30	0.0
Methyl mercury phosphate*	Natural	13	323	3.9	2	296	0.7

* *Trade name = Leytosan P.*

A marked reduction in severity of disease is again shown in the natural soil as compared with the sterilized soil. Comparing the treated and untreated series, it is evident that much of the inoculum naturally infesting this seed sample is surface-borne. However, it would appear that some is internally-borne. It would hardly be expected, for instance, that any surface-borne inoculum would survive the alcohol-flame treatment, yet 6.3% of the seedlings in sterilized soil became infected. It would appear that these were derived from seed bearing internally-borne inoculum of *Polyspora lini*. The alcohol-treated seed was evidently injured considerably by the treatment, since out of 400 seeds sown in unsterilized soil only 30 produced seedlings. None of these, however, showed infection. In the case of the seed treated with methyl mercury phosphate good germination was obtained, yet only 3.9% of the plants in sterilized soil became infected. Comparing this with 0.7, the percentage of infected plants from similarly treated seed sown in natural soil, we find a small but significant difference. The χ^2 value here is 5.67, which lies between the 5% and 1% points. If we assumed that all of the externally-borne inoculum was destroyed by the treatment of the seed with methyl mercury phosphate, then we might conclude that the micro-organisms of the unsterilized soil had an inactivating effect on some of the remaining internal inoculum.

In order to obtain further data on treated and untreated seed, another experiment was conducted. In this experiment, naturally infested four-year-

old Bison seed was used. Instead of the alcohol treatment used in the previous experiment, a formaldehyde treatment was substituted. This consisted in immersing the seed for two minutes in a 1-320 solution of commercial formaldehyde in water and air drying it before seeding. Another series was treated with methyl mercury phosphate at the rate of one ounce per bushel as in the previous experiment. The treated samples, together with untreated checks, were then sown in sterilized and natural soil. Ten replicates were used in this experiment and 50 seeds per pot were sown. The results are given in Table III.

TABLE III

FURTHER DATA ON THE COMPARATIVE SEVERITY OF THE BROWNING DISEASE OF BISON FLAX IN SEEDLINGS FROM TREATED AND UNTREATED SEED IN STERILIZED AND NATURAL SOIL

Seed treatment	Sterilized soil			Natural soil		
	Number of seedlings		Percentage diseased	Number of seedlings		Percentage diseased
	Diseased	Healthy		Diseased	Healthy	
None	65	390	14.3	3	290	1.0
Methyl mercury phosphate	32	430	6.9	2	432	0.5
Formaldehyde 1-320	17	374	4.3	0	83	0.0

It is evident again from these data that something in the natural soil has had a dominant effect in reducing infection to a minimum. The differences in severity of disease between plantings in the sterilized and natural soil are highly significant in the untreated and methyl mercury phosphate series as measured by the χ^2 test, but not in the formaldehyde series. The poor germination in the latter, especially in the natural soil, is no doubt partly responsible for this. In the sterilized soil, both methyl mercury phosphate and formaldehyde reduced infection significantly, but in natural soil this was not the case, since the differences between the treated and untreated series were so small. It is interesting to note from the results of this experiment that the severity of infection was reduced more by antibiotic action than by seed treatment with either fungicide.

In view of the fact that the formaldehyde treatment in the previous experiment was not very satisfactory, owing to the wetting of the seed and the adverse effect on germination, it was thought advisable to use another liquid treatment. Mercuric chloride in combination with alcohol was chosen and used in another experiment, the results of which are given in Table IV. Naturally infested Bison seed was again used. In the treated series the seed was dipped in 70% ethyl alcohol, then immersed for one minute in 1-1000 mercuric chloride solution, then dipped again in 70% alcohol and finally spread on a fine wire screen to dry.

Unfortunately, the severity of infection of the seedlings from the untreated seed in sterilized soil was lower than in previous experiments. The alcohol-

mercuric chloride treatment apparently destroyed approximately the same amount of inoculum as the soil micro-organisms. It would be expected that this treatment would destroy surface-borne inoculum on the seed. If so, the seed used must have carried a very considerable amount of internal inoculum, since on plating, the treated seed yielded almost as high a percentage of infestation as untreated seed. The differences in amount of infection are

TABLE IV

COMPARATIVE SEVERITY OF THE BROWNING DISEASE OF BISON FLAX IN SEEDLINGS FROM SEED TREATED WITH ALCOHOL-MERCURIC CHLORIDE AND UNTREATED SEED IN STERILIZED AND NATURAL SOIL

Seed treatment	Sterilized soil			Natural soil		
	Number of seedlings		Percentage diseased	Number of seedlings		Percentage diseased
	Diseased	Healthy		Diseased	Healthy	
None	32	429	6.9	4	240	1.6
Alcohol-mercuric chloride	9	416	2.1	1	268	0.4

significant when one compares untreated seed in sterilized soil with untreated seed in natural soil or with treated seed in sterilized soil, the χ^2 values being 8.19 and 10.59, respectively. The lowest percentage of infection, namely, 0.4%, was obtained when both seed treatment and the micro-organisms of the natural soil were allowed to act, but the differences between this figure and 2.1 for the treated seed in sterilized soil and 1.6 for the untreated seed in natural soil are insignificant. Hence it is not possible to conclude from this experiment that the micro-organisms of the soil had a significant effect on the internal inoculum, nor that seed treatment with mercuric chloride reduced infection in natural soil.

RESULTS WITH *Colletotrichum lini*

In several experiments on the flax Anthracnose disease conducted in another connection, it was noticed that seedling infection of seedlings from diseased seed was consistently much less severe in natural soil than in the same soil following steam sterilization. Table V, for example, gives data from three

TABLE V

COMPARATIVE SEVERITY OF THE ANTHRACNOSE DISEASE OF FLAX CAUSED BY *Colletotrichum lini* IN STERILIZED AND NATURAL SOIL

Experiment	Soil temperature, °C.	Percentage of infected seedlings	
		Sterilized soil	Natural soil
1	12	53	14
2	17	71	18
3	24	75	19

separate experiments, each conducted at a different temperature. All show the same reduced severity of the disease in the natural as compared with the sterilized soil.

The seed used in these experiments on *Colletotrichum lini* was artificially inoculated. As will be noted, much more severe infection was obtained than with *Polyspora lini*. While infection was greatly reduced in natural soil, it was not lowered to the extent that it was in the experiments on the Browning disease. Definite evidence, however, was obtained regarding the identity of one of the inactivating organisms, since a bacterium was found which caused rapid disintegration of the conidia of *Colletotrichum lini*. This bacterium, if present in the soil, could readily destroy surface-borne spores on the seed and thus reduce the severity of infection.

Experiments with Covered Smut Fungi of Cereals

Since the cereal smut fungi are among the best known seed-borne pathogens, it was thought that similar experiments to those reported with the flax pathogens should be made with them. These have been undertaken with the covered smut pathogens affecting wheat, oats and barley, but have not as yet been completed. However, it seems desirable at this time to refer briefly to the preliminary results with the bunt fungi of wheat. Plantings of artificially infested seed in sterilized and natural soil have to date not shown the consistent behavior that we have demonstrated for the flax pathogens. Instead of the severity of infection being reduced, the data obtained so far show higher infection in the natural soil in some cases, but as this has not so far been consistently in the same direction it seems advisable to check the results by further experiments. These will be discussed in another paper to be published soon.

Discussion

While chemical and physical changes in the soil are brought about by steam sterilization, the reduction in disease severity noted in the experiments reported with flax pathogens seems best explained on biological grounds. In support of this hypothesis it has been noted that certain soil bacteria have a marked inhibitive action on the flax pathogens investigated, which is in agreement with the findings of Novogrudski *et al.* (2). This action has been studied by one of us* as it affects *Colletotrichum lini* and will be reported on in a subsequent paper. Soil sterilization would obviously destroy such antibiotic organisms. Hence, unless the products remaining after sterilization could act as efficiently as the living organisms, which does not appear to be the case, at least for certain antibiotic organisms, one would expect more severe infection in sterilized soil.

Since preliminary results with smut fungi indicate that some of them are not inactivated to the same degree as the flax pathogens in natural soil, it

*CAMPBELL, J. A. Studies on anthracnose diseases of grains and grasses in Alberta with special reference to flax anthracnose caused by *Colletotrichum lini* (West.) Toch. M.Sc. Thesis, University of Alberta. 1934.

appears that different seed-borne pathogens are affected differently by the soil micro-organisms, as might be expected. Seed treatment may consequently be more important for the control of some pathogens than for others. Obviously, the influence of soil micro-organisms on seed-borne plant pathogens needs further investigation.

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THE NITROGEN DISTRIBUTION IN ALFALFA HAY CUT AT DIFFERENT STAGES OF GROWTH[1]

By M. F. Clarke[2]

Abstract

Analyses were made of alfalfa hays cut at six different stages of growth during the course of one growing season. For the determination of the soluble forms of nitrogen encountered in this study, the method developed by Wasteneys and Borsook for the fractional analysis of incomplete protein hydrolysates was applied. This procedure ensured the securing of more definite information concerning the exact gradations of protein synthesis and breakdown than is obtained by the usual amino and amide nitrogen determinations.

The data for total nitrogen show a relatively steady decrease with advancing maturity of the harvested material. Also, the second and third cuttings of hay exhibit a distinctly higher total nitrogen content than the first cutting.

Total soluble nitrogen tends to exhibit considerable fluctuation throughout the growing period. Cuts made later in the season show a progressive decrease in the proportionate amounts of this fraction. Particularly striking is the finding that, in all cuttings, total soluble nitrogen tends to be present in very large amounts during the early part of flowering.

Results obtained from the application of the method of Wasteneys and Borsook make possible the presentation of a relatively clearly defined pictur of protein synthesis and degradation throughout the growing period. These data further strengthen the assumption that the period between bud formation and the commencement of flowering represents a very critical stage in the life history of the plant.

Introduction

Numerous research workers have demonstrated that legumes and grasses cut at different stages of growth produce hays which differ markedly not only in total but also in digestible protein. The corresponding studies suggest a probable occurrence of significant variations in the actual distribution of different forms of nitrogen.

The lack of definite information respecting the distribution of intermediate nitrogen compounds is undoubtedly due to the fact that studies on nitrogen metabolism in plants have been confined largely to the distribution of those relatively simple forms of nitrogen which are obtained after the hydrolysis of aqueous extracts of plant tissues. The usual mode of study is well demonstrated by the works of Chibnall (1), Thomas (8), Vickery and Pucher (9), Stuart and Appleman (7), Richards and Templeman (6), and Orcutt and Wilson (4).

Since it is highly probable that protein synthesis and breakdown occur in several stages, giving rise to compounds of varying complexity, it seemed reasonable to assume that a method capable of defining such more or less specific intermediate products should provide valuable information in addition to that gained from the usual procedure. With this in view, a study of the method developed by Wasteneys and Borsook (10) for the fractional analysis of incomplete protein hydrolysates was undertaken and made the basis for the subsequent report.

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Contribution from the Department of Agronomy, The University of British Columbia, Vancouver, Canada.

[2] *Graduate student, Department of Agronomy, The University of British Columbia, Vancouver.*

The Wasteneys and Borsook method defines the exact nature and amount of the specific protein decomposition fractions (proteose, peptone, and subpeptone) following the breakdown of protein by specific enzymes. The procedure involves the precipitation of protein by trichloracetic acid; of metaprotein by careful adjustment of the hydrogen ion concentration; of proteoses by saturation with sodium sulphate at 33° C.; of peptones by tannic acid under definitely controlled conditions; and the determination of the residual amino acids and simpler peptides by a modification of the alcohol precipitation method.

Experimental Methods

In order to determine the suitability of the method of Wasteneys and Borsook for use in plant nitrogen studies, a study of the nitrogen distribution in alfalfa hay cut at different stages of growth was undertaken.

The alfalfa used in this investigation was a strain of Ontario Variegated (Medicago media) designated as Ottawa, No. 176. The seed was sown originally on duplicate plots in 1933. Three cuttings of hay were taken during each of the succeeding seasons of 1934 and 1935, while the 1936 material was used for this study.

Samples for analysis were harvested at the following stages of growth: (1) Seedling; (2) Pre-bud; (3) Bud; (4) Early flowering (1/10-bloom); (5) Full bloom; (6) Maturity.

The growing period during which samples were taken was of sufficient duration to permit of taking second and third cuts from the same plots at corresponding stages of growth. The entire aerial portions of the plants were used for each examination. Immediately after cutting, the samples were transferred to special drying trays and taken indoors. This enabled the drying to take place at a relatively rapid rate without any appreciable loss in color or wastage of leaves. The resultant product was a hay of slightly better quality than alfalfa hay made under the most ideal field conditions.

Analytical Methods

Prior to analysis, all samples were ground with the aid of a small hand-operated corn mill to a degree of fineness approximating that of the dehydrated alfalfa meal of commerce. In order to facilitate grinding, the samples were subjected to a preliminary drying in an electric oven at a temperature of 45° C. for 24 hr.

For the determination of dry matter contained in the ground material, three aliquots of approximately 0.5 gm. were placed in tared aluminium crucibles, weighed and dried to constant weight in a water-jacketed oven at a temperature of 98° C.

Total nitrogen was determined on quadruplicate one-gram samples by the Gunning modification of the Kjeldahl method.

Fractionation of Soluble Nitrogen

The soluble nitrogenous material was extracted from the finely ground alfalfa by means of boiling water, according to the method of Davidson,

Clark and Shive (2). All extractions were performed in duplicate immediately before analysis.

Four 5-cc. portions of the water extract (filtrate 1) were analyzed for total soluble nitrogen by the Kjeldahl method. For verification purposes, the nitrate modification of the Kjeldahl method, as proposed by Ranker (5), was followed, but the results obtained were no higher than those from the standard Kjeldahl procedure. In addition, semi-quantitative tests with acidified diphenylamine reagent failed to reveal even traces of nitrate nitrogen in the extracts of any of the samples.

For the determination of the various nitrogenous fractions (protein, proteose, peptone, and sub-peptone) the procedure used was that of Wasteneys and Borsook (10), as modified by Eagles and Sadler (3) in the study of nitrogen distribution during cheese-ripening.

Experimental Results

The analytical results obtained during the course of this investigation are summarized in Table I. The results for total nitrogen and total water-soluble nitrogen are expressed as percentage of the absolute dry weight of the material. The soluble nitrogenous fractions (W & B) are expressed as percentage of the total water-soluble nitrogen of each respective sample.

TABLE I

THE NITROGEN DISTRIBUTION IN ALFALFA HAY CUT AT DIFFERENT STAGES OF GROWTH

Stage of growth	Cutting no.	Total N(%)*	Total water-soluble N(%)*	Soluble protein N†	Proteose N	Peptone N	Sub-peptone N
Seedling	Cutting No. 1	4.530	1.867	21.65	7.92	8.16	62.27
Pre-bud	Cutting No. 1	2.885	1.275	17.17	6.69	8.47	67.67
	Cutting No. 2	4.333	1.923	29.74	6.92	9.95	53.39
	Cutting No. 3	3.815	1.201	17.11	8.23	7.94	66.64
	Cutting No. 4	3.152	1.292	20.67	9.91	8.33	61.10
Bud	Cutting No. 1	2.375	1.060	32.90	11.71	12.38	43.01
	Cutting No. 2	3.844	1.418	17.01	9.03	21.55	52.41
	Cutting No. 3	3.665	1.103	34.22	8.99	6.30	50.49
Tenth-bloom	Cutting No. 1	2.777	1.463	19.08	9.67	18.50	52.75
	Cutting No. 2	2. 61	1.014	8.80	11.99	12.01	67.20
	Cutting No. 3	3.726	1.416	23.43	14.79	14.10	47.68
Full bloom	Cutting No. 1	2.276	0.686	24.08	12.19	13.84	49.89
	Cutting No. 2	2.660	0.861	15.94	20.41	11.69	51.96
Maturity	Cutting No. 1	2.223	0.774	11.07	20.28	15.81	52.85

* *Total nitrogen and total water-soluble nitrogen are expressed as percentage of moisture-free material.*

† *Results for the soluble nitrogenous fractions (soluble protein, proteose, peptone and sub-peptone N) are expressed as percentage of the total soluble nitrogen of each respective sample at the stages of growth here defined.*

Discussion

Total Nitrogen

An examination of the data in Table I reveals a steady decline in total nitrogen with advancing maturity of the harvested material. This decline, as might be expected, is not quite uniform when the different cuttings at each stage of growth are compared with the corresponding cuttings at subsequent stages of growth. Furthermore, the data obtained with respect to total nitrogen are rather limited and do not permit of drawing any far-reaching conclusions. However, these results are similar to those obtained by Woodman and Evans (11) in a comprehensive study dealing with the nitrogen content of alfalfa. These investigators observed a marked drop in the nitrogen content of both the leaf and stem with advancing maturity of the plants.

Woodman and Evans (11) reported, among others, that the second and third cuttings of alfalfa hay were of a distinctly leafier character than the first cutting. This, in turn, accounts for a higher total nitrogen content in each subsequent cutting at each stage of growth, since at any one stage the nitrogen content of the alfalfa leaf is approximately double that of the stem. It deserves to be noted that the data presented in Table I bear out this contention as regards all but one stage of growth—that of the pre-bud stage.

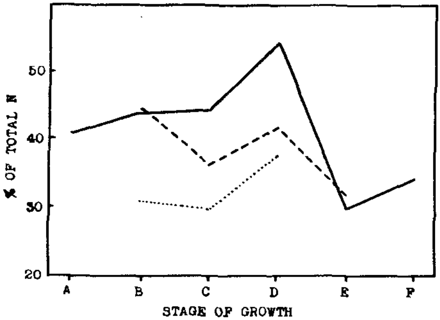

FIG. 1. *Total soluble nitrogen of alfalfa hay cut at different stages of growth. A—seedling, B—pre-bud, C—bud, D—tenth-bloom, E—full bloom, F—maturity. Cutting No. 1———, No. 2- - - -, No. 3· · · · · ·.*

Total Soluble Nitrogen

The total soluble nitrogen results are expressed graphically in Fig. 1. In order to emphasize the fluctuations between each stage of growth, the results are plotted as percentage of the total nitrogen of each respective sample.

It is interesting to note that in most cases the graphs tend to suggest a decrease in the proportionate amount of total soluble nitrogen at each stage

of growth with each subsequent cutting. Attention should also be drawn to the fact that the proportion of soluble nitrogen is distinctly higher at tenth-bloom than at budding, in all three cuttings, and that in the first and also in the third cutting, soluble nitrogen attains its maximum quantity at the tenth-bloom stage. Judging from these results, it would seem reasonable to conclude that a certain amount of protein degradation takes place for the purpose of creating a readily translocated supply of simpler nitrogenous compounds required for the process of flowering. On the other hand, it might also suggest that a more rapid intake of nitrogenous material occurs at this time, which may be held in a soluble form to meet the needs of flower formation. The fact that the total nitrogen content of the second cutting at tenth-bloom is lower than at the preceding stages of growth bears out the first contention, whereas the increase at tenth-bloom over the bud stage in the first and third cuttings might substantiate the second assumption.

The marked decrease in the proportionate amount of total soluble nitrogen from early to late flowering indicates that the excess of soluble nitrogenous material has been utilized in the normal plant processes, such as preparation for seed setting and increases in fibre content and stem growth. The slight rise in soluble nitrogen at the time of maturity might be indicative of protein degradation in order to translocate nitrogen to the seeds already formed. On the other hand, it may be caused by a general breakdown following the cessation of all growth processes.

Soluble Protein Nitrogen, Proteose Nitrogen, Peptone Nitrogen, and Sub-peptone Nitrogen

An examination of the data presented in graph form (Fig. 2) shows very clearly that soluble protein, proteose, and peptone individually represent a relatively small proportion of the total soluble nitrogen, while sub-peptone constitutes almost half of the soluble fraction.

Soluble protein tends to be greater in amount than either proteose or peptone at nearly all stages of growth, and the curve for this fraction shows more pronounced fluctuations. Since proteoses are considered to be only slightly below proteins in complexity, it might be expected that the trends for the two would have a tendency to approximate each other. This relation, however, does not hold, except in two instances.

In the first cuttings (Fig. 2, I), proteose follows a trend similar to that exhibited by soluble protein up to the time of full bloom, although the actual amount of proteose is appreciably less. Following the period of flowering, the situation is reversed, and the quantity of proteose is almost double that of soluble protein. The third cuttings follow a similar course. The second cuttings (Fig. 2, II) show an inverse relation between soluble proteins and proteoses from pre-budding to tenth-bloom. From tenth-bloom to maturity, the relation is fairly constant, except that the order of magnitude is altered.

As regards peptone nitrogen, it may be noted, in the first cuttings, that the curves for proteose and peptone nitrogen (Fig. 2, I) are of practically the same order throughout the growing period. At tenth-bloom there appears

to be an appreciable difference in favor of higher peptone content, while the graphs would appear to suggest slightly less peptone nitrogen at the time of maturity. It should be pointed out, too, that in many instances the actual trends of peptone are inclined to be the reverse of those for protein and proteose.

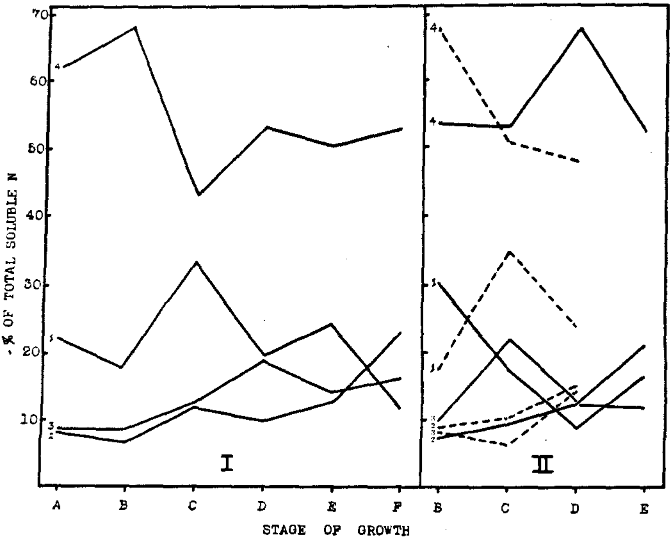

FIG. 2. *Distribution of soluble protein (1), proteose (2), peptone (3), and sub-peptone (4), nitrogen. I—Cutting No. 1. II—Cutting No. 2——, Cutting No. 3- - - - - -. Stages: A—seedling, B—pre-bud, C—bud, D—tenth-bloom, E—full bloom, F—maturity.*

In the third cuttings (Fig. 2, II) the graph for peptone is again of approximately the same order as that of the first cuttings, but in this case the peptone content appears to be slightly lower than that of proteose nitrogen. The graph covering the second cuttings (Fig. 2, II) does not conform to the other curves, in that it appears to demonstrate a definite negative correlation between proteose and peptone at the stages of growth from which comparisons were taken. This also holds true when a comparison is made between soluble protein and peptone.

As already noted, the sub-peptone fraction, in most instances, represents a greater proportion of the total soluble nitrogen than that of soluble protein,

proteose, and peptone combined. Since sub-peptone includes peptides and free amino acids of lesser complexity than peptone, the curves for this fraction tend to compensate for the fluctuations noted in regard to protein, proteose, and peptone. It is significant that sub-peptone should be at its highest level in the early stages of growth, while the more complex forms of nitrogen—protein, proteose, and peptone—are to be found in lesser amounts at the corresponding stages. This would suggest that nitrogen intake has been more rapid than protein synthesis. However, when flower buds have appeared at the time of the first cuttings, sub-peptone has decreased to the lowest level observed in any part of the cycle, thereby showing that, at later stages, synthesis has been sufficiently rapid to surpass nitrogen intake. Coincident with this major depression in the sub-peptone curve, one may note a peak in the protein curve and the lesser rises in the proteose and peptone curves. Between the first appearance of flower buds and the one-tenth bloom, sub-peptone and peptone increase, whereas the more complex compounds (proteins and proteoses) exhibit a corresponding decrease. The behavior of these fractions during the period of flowering would suggest that a breakdown of relatively complex nitrogen compounds takes place in order to create a readily translocated supply of nitrogenous material to meet the needs of flower formation.

In the second cuttings, sub-peptone represents a much lower proportion of the soluble nitrogen during the early stages of growth than in the first cuttings, while soluble protein has reached its peak at this time, suggesting the possibility that synthesis may be more rapid following the removal of the first growth. During the early part of flowering (1/10-bloom), an extremely high proportion of sub-peptone, together with low protein, substantiates previous assumptions relative to bloom formation.

In the third cuttings, the distribution of the four fractions under discussion closely parallels that prevailing in the first cuttings. However, the behavior of sub-peptone between budding and the early part of flowering is the reverse of that encountered in the first and second cuttings. This fact does not completely invalidate the assumptions made with regard to the need for an abundant supply of relatively simple forms of nitrogen to meet the requirements of blooming. On the other hand, it suggests the possibility that an abundance of slightly more complex compounds, such as proteoses and peptones, may meet this requirement. The fact that proteoses and peptones increase, furnishes further evidence in support of this assumption.

Conclusions

Primarily, the object of the present investigation was to determine if the method of Wasteneys and Borsook (10) could be applied advantageously to the study of nitrogen distribution in plant material. It was realized, at the time, that the information gained from its successful application would provide a useful complement to the knowledge already obtained in the course of other methods of analysis.

The results obtained have not been presented with the idea that they are conclusive or that the objects of the investigation have been fully realized. Since the figures for the analysis were secured directly from the existing material without further modification through hydrolysis, and since the distribution of soluble nitrogen has not been disturbed in any way, but has been studied in its original form, one may be justified in contending that the data do possess a definite significance, for every nitrogen fraction studied. It should be noted, too, that the amounts of many of the fractions are extremely small, and that the relation existing between them at any one time is much more complex than that encountered at one stage in the hydrolysis of pure proteins by specific enzymes.

Considering the fact that marked differences occur between the varying stages of growth examined, one is led to conclude that further valuable information might be gained about the nitrogen metabolism of the alfalfa plant if a study of this subject were conducted on fresh samples collected at shorter intervals throughout the growing season. In this way the exact gradations of synthesis and breakdown could be more clearly observed.

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THE INFLUENCE OF BIOS ON NODULE BACTERIA AND LEGUMES[1]

B. INFLUENCE OF CRUDE BIOS PREPARATIONS ON ACID PRODUCTION BY STRAINS OF *RHIZOBIUM TRIFOLII*

By D. G. Laird[2] and P. M. West[3]

Abstract

Certain components of Wildiers' Bios complex, fractionated and concentrated according to the procedure of Miller and co-workers, were found capable of replacing the stimulative action of yeast extract on strains of *Rhizobium trifolii*, as measured by acid production. Bios I was inactive, while Bios II B, V, and II A possessed definite activity, the potency of the fractions increasing in the order named. Moreover, the ability of these fractions to increase hydrolysis of urea by urease was in direct proportion to the stimulative effect exerted by them on the *Rhizobia*. These effects could not be brought about in synthetic media by the addition of crystalline vitamin B_1, nicotinic acid, uracil, choline, β-alanine, carnosine, β-indole acetic and β-indole butyric acids, glutathione, cysteine and vitamin C.

Introduction

A study of the influence of the Bios complex on nodule bacteria, alone and in association with the host, was undertaken in the belief that the information thereby obtained might contribute to a more complete understanding regarding conditions necessary to bring about nitrogen fixation. While the host plant behavior of red clover seedlings in the presence of growth factors was reported upon in a previous paper (Laird and West, 9), attention at this time is being focused on the response of the *Rhizobia* themselves to such activators as might possibly contribute to optimum bacterial activity. Aside from the investigations of Allison, Hoover and Burk (1) and Allison and Hoover (2–4), relatively little has been reported so far concerning this phase of the physiology of legume bacteria.

It was demonstrated by Thorne and Walker (15), and recently confirmed in this laboratory, that in a medium of a favorable oxidation-reduction potential, *Rhizobium trifolii* does not appear to require for mere maintenance of life any organic substance other than a pure fermentable carbohydrate. It should be remarked, however, that such suitable carbohydrates alone provide conditions which, at best, result in only meagre growth. Obviously some other factor or factors are required to bring about the luxuriant growth produced by yeast extract, and it is the additional purpose of this paper to present the results of a preliminary study regarding the nature of the specific stimulatory substances involved. The materials referred to herein as "activators" are understood to mean organic substances which, without being essential

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[2] *Associate Professor, Department of Agronomy (Soils), The University of British Columbia, Vancouver, Canada.*
[3] *Carnegie Scholar, Department of Agronomy (Soils), The University of British Columbia, Vancouver, Canada.*

to the life of the cell, in minute amounts are capable of exerting a marked stimulatory influence on certain metabolic processes.

Acid Production as a Measure of Stimulation

The problem of establishing a convenient method for estimating the metabolic activity of *Rh. trifolii* presented considerable difficulty. It has been frequently observed that, accompanying the stimulating effect of yeast extract on the growth of the organism, an increase of acidity occurs in the medium, which is more marked in some strains than in others. It was believed that if the selection of strains was made with a view to obtaining types capable of relatively high acid production, a gradation value representative of their activity could be arrived at by direct titration of the cultures. The possibility that cell multiplication and acid production might be controlled by separate factors was fully realized, but for this preliminary investigation of the response of the organisms to activators, it was decided to limit the scope of the survey largely to those factors which stimulate acid production to a smaller or greater extent.

Selection of *Rhizobium trifolii* Strains and Development of Basal Medium

Approximately 60 strains of *Rh. trifolii*, from both stock and freshly isolated cultures, were examined for acid-producing ability in the standard yeast-water-mannitol medium. From this group two strains were selected, checked for purity, and adopted for the study. One, Strain 22B, was known by its previous record to be distinctly efficient in symbiotic nitrogen fixation, while the other, Strain 202, on the contrary, was decidedly inefficient in this respect.

The basal medium adopted consisted of 10 gm. mannitol, 0.5 gm. K_2HPO_4, 0.2 gm. $MgSO_4$, 0.1 gm. NaCl, and a trace of $FeCl_3$, per litre of distilled water. Bromthymol blue indicator was added and the media adjusted to pH 7.0. Titration of the cultures was performed, using freshly prepared N/200 NaOH, and the results were expressed as ml. N/4 acid produced per litre.

Since preparations of Wildiers' Bios used in this study contained a certain amount of nitrogen, it was considered advisable to determine whether common inorganic nitrogenous compounds might exert any appreciable effect. Ammonium chloride as a source of nitrogen for the selected strains of *Rh. trifolii* was therefore tried in varying concentrations from 5% to 0.00005% actual nitrogen. Both growth and acid production, although slight in all cases, were most marked below 0.001% nitrogen, but the organisms continued to grow and produce acid in a medium containing up to 0.05% nitrogen. In no instance did the presence of ammonium chloride alone raise the acid production beyond 3 ml. N/4 acid per litre in 48 hr. Sodium nitrate was tested as above, with essentially the same results.

In studying the influence of the simpler forms of organic nitrogen, 12 amino acids (Eastman's products) as well as urea, asparagine, carnosine and uracil were employed. Sufficient material was added in each case to bring the

nitrogen contents of the media to 0.0005%. The organisms were capable of utilizing all the above forms of nitrogen, but with varying degrees of efficiency, as measured by acid production. In general, valine, leucine, histidine, arginine, cysteine, urea, uracil and carnosine produced much the same response as ammonium chloride, while glycine, alanine, phenylalanine, proline, tryptophane, lysine and asparagine proved to constitute relatively inferior nitrogen sources. It is important to note that none of these cultures showed an acid production of 3 ml. or over after two days, whereas acid was produced far in excess of this figure in all media enriched by yeast extract.

In order to make the study of nitrogen sources more complete, the response of *Rhizobia* to two kinds of purified protein was investigated—namely, sodium caseinate and edestin (Difco products). Although these strains of *Rhizobia* are not generally considered as proteolytic bacteria, they did, however, demonstrate their ability to utilize the two proteins very slowly, with the production of small amounts of acid, as was found with the simpler sources of nitrogen.

From the above studies it was concluded that, since *Rhizobium trifolii* requires only small amounts of nitrogen—five to ten parts per million being optimum for growth in fluid culture—and since relatively little nitrogen may even exercise an inhibitory effect, best results would be obtained through the use of a nitrogen-free basal medium with impure preparations of activators.

Influence of Wildiers' Bios Fractions on Activity of *Rhizobium trifolii*

It was deemed possible that the semi-purified fractions of the Bios complex which contain activators necessary for the development of yeasts might also prove to contain such stimulatory substances as are required for maximum activity of the *Rhizobia*. If such concentrates of Bios could replace yeast extract, it was believed that this finding would be of significance in relating

TABLE I

Influence of Bios II A on acid production of *Rhizobium trifolii*

Addition to basal media	Strain 22B		Strain 202	
	24 hr.	48 hr.	24 hr.	48 hr.
Bios II A, 2%	3.6*	8.4	5.8	14.0
Bios II A, 1%	4.1	14.0	6.6	15.2
Bios II A, 0.8%	4.6	10.2	6.6	12.9
Bios II A, 0.4%	6.0	10.2	8.1	11.1
Bios II A, 0.2%	2.4	8.0	2.8	9.2
Bios II A, 0.1%	2.2	7.0	2.4	6.8
Bios II A, 0.05%	2.0	4.0	2.4	6.0
Bios II A, 0.01%	0.6	0.6	0.2	2.0
Yeast extract, 10%	4.1	9.0	3.6	6.0
NH_4Cl nitrogen, 0.001%	0.6	1.6	0.6	1.9
Nitrogen-free control	0.0	0.6	0.2	0.8

* *Ml. N/4 acid produced per litre.*

the activators for the *Rhizobia* to a group of known growth factors, one of which has been isolated by Kögl and Tönnis (8).

The Bios fractions were prepared from tomato juice by the recent Miller method (11). Crude Bios II A, so prepared, contained 1.01% nitrogen. This material was used as enrichment for the basal medium in concentrations from 2% to 0.01%. As may be deduced from Table I, the response of the organisms to Bios II A is definitely significant, and this part of the Bios complex is evidently able to replace yeast extract as an activator of acid production.

Since it had also been observed in previous experiments that the presence of the Bios fractions stimulated the production of ammonia in a urea medium, it was thought possible that the pure enzyme would therefore respond to the active constituents of the Bios fraction in the same manner as the organism itself. If this were so, a more rapid and convenient assay might be available. To test this possibility, purified urease (B.D.H. blood analysis tablets) was used.

The basal medium for the urease test was the same as that used for bacterial studies, except that 2 gm. urea was substituted for 10 gm. mannitol per litre. To 5-ml. amounts of this medium, the active extract, carefully neutralized, was added in concentrations from 0.2% to 15%. After adding cresol red indicator, all tubes were adjusted to pH 7.2 with $N/200$ sodium hydroxide or hydrochloric acid. Then 1 ml. of a 1 : 10 dilution of a standard urease tablet was added to each tube, and after shaking, the mixture was incubated at 28° C. for 2 hr. At the end of this time the ammonia produced was titrated with $N/200$ hydrochloric acid and the results expressed as increase in ml. $N/4$ ammonium hydroxide per litre over the control. The results of such a test with the Bios II A fraction are presented in Table II.

TABLE II

INFLUENCE OF BIOS II A ON UREASE ACTIVITY

Per cent Bios II A	0.2	0.4	0.8	1	2	4	8	10	15
Ml. $N/4$ NH_4OH per litre in excess of control	5.3	6.0	5.8	5.0	4.2	3.7	1.6	0.8	0.6

These data indicate that the Bios II A fraction operates as an activator also for the urease enzyme system. The optimum concentration of Bios II A in the urease test is the same as for strains of 22B and 202 when measured by their acid production at 24 hr. Although this "activation" may be due to the heavy-metal-binding capacity of the organic extracts, it is significant, nevertheless, that the potency of an unknown active fraction to stimulate acid production of *Rhizobia* may be foretold, and the optimum concentration accurately determined by the urease test. Whatever substances in the fractions are active in bringing about a more rapid hydrolysis of urea by the enzyme would appear to be the same as the stimulatory factors for *Rhizobia*, or else

to be present in direct proportion. In later work undertaken in connection with a further purification of the Bios II A fraction, the urease test proved to be a valuable and convenient means to determine the course of activity in the isolation process.

The corresponding Bios II B fraction, containing 0.71% nitrogen, was tested on urease and on the selected strains of *Rh. trifolii* in much the same range of concentrations as Bios II A. The results given in Tables III and IV indicate that Bios II B also contains ingredients capable of activating the organisms and the urease enzyme, thus replacing the effect of yeast extract. In general, Bios II B is considerably less active than Bios II A, as shown by the different response of the bacteria and confirmed by the behavior of urease.

TABLE III

INFLUENCE OF BIOS II B ON ACID PRODUCTION OF *Rhizobium trifolii*

Addition to basal media	Strain 22B		Strain 202	
	24 hr.	48 hr.	24 hr.	48 hr.
Bios II B, 4%	3.0*	7.4	3.2	7.0
Bios II B, 2%	3.0	7.2	3.2	7.0
Bios II B, 1%	3.0	7.2	3.4	7.0
Bios II B, 0.8%	3.3	7.6	3.0	7.0
Bios II B, 0.4%	2.3	6.0	2.4	6.4
Bios II B, 0.2%	2.1	2.6	2.4	4.5
Bios II B, 0.1%	1.4	1.6	1.9	2.4
Bios II B, 0.05%	0.8	1.4	0.8	1.8
Bios II B, 0.01%	0.4	0.7	0.5	1.0

* *Ml. N/4 acid produced per litre.*

TABLE IV

INFLUENCE OF BIOS II B ON UREASE ACTIVITY

Per cent Bios II B	0.2	0.4	0.8	1	2	4	8	10	15
Ml. *N*/4 NH_4OH per litre in excess of control	0.8	0.9	2.0	2.4	2.8	3.3	3.5	3.8	3.6

Bios V has been recently studied by Elder (6), who found it essential for certain strains of yeasts. In contrast to the other constituents of the Bios complex, this fraction is sensitive to heat in the presence of alkali.

A Bios V concentrate was prepared according to Elder's procedure, to determine whether or not this substance is necessary for maximum activity of *Rh. trifolii*. The fraction as prepared contained 0.085% nitrogen. The stimulative effect of Bios V on *Rhizobium trifolii* and urease action is apparent from Tables V and VI. It is also apparent that the maximum activating power of Bios V lies between that of Bios II A and Bios II B. This relation has held true for all Bios V fractions that have been prepared.

TABLE V

INFLUENCE OF BIOS V ON ACID PRODUCTION OF *Rhizobium trifolii*

Addition to basal media	Strain 22B		Strain 202	
	24 hr.	48 hr.	24 hr.	48 hr.
Bios V, 4%	8.6*	12.1	1.4	13.0
Bios V, 2%	10.2	11.9	2.4	12.0
Bios V, 1%	7.8	9.5	1.5	10.0
Bios V, 0.8%	6.9	8.6	1.4	9.7
Bios V, 0.4%	4.3	9.4	1.4	10.4
Bios V, 0.2%	2.4	3.6	0.8	5.8
Bios V, 0.1%	0.8	1.0	0.0	1.8
Bios V, 0.05%	0.0	1.0	0.0	1.0
Bios V, 0.01%	0.0	0.5	0.0	0.0

* *Ml. N/4 acid produced per litre.*

TABLE VI

INFLUENCE OF BIOS V ON UREASE ACTIVITY

Per cent Bios V	0.2	0.4	0.8	1	2	4	8	10	15
Ml. *N*/4 NH_4OH per litre in excess of control	1.6	1.7	3.2	3.2	4.6	4.0	4.0	3.4	2.1

Since all the substances of the Bios complex occur together in natural plant and yeast extracts, an effort was made to determine the ideal proportion of the Bios fractions required for maximum activity. In no instance was it found that the results produced by combining various sub-optimal amounts of two or more fractions were greater than would be expected from the additive effect, and frequently they were somewhat less. Bios I (inositol), which, used alone, was without effect on *Rhizobia*, was also tested with varying proportions of the other three fractions, resulting in either no effect or a slight depression of activity. The observations of Miller *et al.* (10), and Eagles *et al.* (5), concerning the "greater than additive" effect of Bios combinations over fractions tested singly, were found to be inapplicable to the strains of *Rhizobia* studied. These workers used yeasts and lactic acid bacteria respectively.

Comparative Stimulation by Bios Fractions and Known Compounds

Since Tatum, Wood and Peterson (14) have shown vitamin B_1 to be essential for the propionic acid bacteria, and James and Knight (7) have demonstrated that *Staphylococcus aureus* is activated by that vitamin in the presence of nicotinic acid, these compounds were investigated as possible activators for *Rhizobia*.

Vitamin B_1 is not so stable to heat as are the members of the Bios complex, but it appears from the work of Schopfer and Jung (13) and Robbins and Kavanaugh (12) that, in the event of its destruction upon sterilization, the

decomposition products might possess activity. Be this as it may, the media were sterilized in such a manner as to avoid destruction of the vitamin. The media were enriched with nitrogen as ammonium chloride. Vitamin B_1 alone, in concentrations ranging from 0.001 to 50 γ per ml. and in combination with nicotinic acid in concentrations from 1000 to 0.2 γ per ml., was used as enrichment for the media. No stimulation of acid production by the organisms could be ascertained.

It was thought possible, however, that, although vitamin B_1 exerted no influence on *Rhizobia* when tested alone, there might be some interrelation between this vitamin and substances of the Bios group, and that an effect of vitamin B_1 might occur only if the two were added together. It appeared that, in combination with sub-optimal amounts of Bios II A or Bios II B, relatively high concentration of the vitamin (1 γ/ml.) produced a small but constant stimulative effect. Since, however, such large amounts were required for only a slight increase in activity, vitamin B_1 cannot be considered a significant activator for the organism.

Among other pure substances tested over a wide range of concentrations and found incapable of replacing the activity of fractions of the Bios group were uracil, choline, β-alanine, carnosine, β-indole-acetic and β-indole-butyric acids, glutathione, cysteine and vitamin C.

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INTERFERTILITY PHENOMENA IN *FOMES PINICOLA*[1]

BY IRENE MOUNCE[2] AND RUTH MACRAE[3]

Abstract

Studies of sexuality in *Fomes pinicola* (Sw.) Cooke based on material from 43 new sources, combined with results already published (1929) have led to the following conclusions: (i) Collections from North America may be divided into two groups, a large Group A, and a small Group B. Monosporous mycelia of any culture in Group A are compatible (mutually fertile) with those of every other member of the group; similarly monosporous mycelia of any member of Group B are compatible with other monosporous mycelia of Group B; but monosporous mycelia of Group A are almost completely incompatible (sterile) when paired with those of Group B. (ii) Collections of European and Japanese origin form a third Group C. These are almost completely compatible with Group A and only partially incompatible with Group B. (iii) In Group B are several isolates of the so-called *Populus* or hardwood form of *F. pinicola* which has been designated at times as a separate species *F. marginatus*. The remainder are, however, the typical coniferous or "red-belt" form.

An earlier publication on the biology of *Fomes pinicola* (Sw.) Cooke (4) contains a review of the literature, notes on the distribution of the fungus including a host list of 91 species, observations on spore germination, cultural characters, effects of variation in temperature and acidity upon mycelial development, mixed cultures and the formation of a line of demarcation, presence of chlamydospores and the production of sporophores in culture, and the macroscopic and microscopic characters of rot caused by this fungus. In addition, the following statements concerning the behavior of paired monosporous mycelia were made:

1. *F. pinicola* is a heterothallic, bisexual (bipolar) species.

2. Monosporous mycelia which had been kept in culture for five years remained in the haploid condition.

3. Many so-called sexual strains or geographical races, which were completely cross-fertile, were found.

4. Monosporous mycelia isolated from sporophores from deciduous hosts were mutually fertile with those from sporophores from coniferous hosts; monosporous mycelia from cultures from France and Sweden were mutually fertile with monosporous cultures from all Canadian sources except one culture from British Columbia (No. 562C). These results, interpreted in the light of the clamp-connection criterion for identity of species, furnish experimental evidence in support of the generally accepted conclusions *(a)* that *F. marginatus* and *F. pinicola* are one and the same species, and *(b)* that the European and American forms of this fungus are identical.

Since that time a large number of cultures from various sources have been investigated. Some of these conclusions may be modified, others amplified, in the light of further experiments.

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Contribution No. 558, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. (Continuing the series of the former Division of Botany.)
[2] *Senior Assistant Agricultural Scientist (Plant Pathology), Ottawa.*
[3] *Assistant Agricultural Scientist (Plant Pathology), Ottawa.*

Materials and Methods

A complete list of the cultures used, and the host and locality from which each came, is given in Table I. In addition to those reported previously (4) there are cultures from 43 new sources. Specimens from the following coniferous and deciduous hosts are included, the figure indicating the number of collections in each case: *Abies balsamea* 1, *A. concolor* 1, *A. grandis* 3; *Picea Engelmanni* 2, *P. glauca* 3, *P. mariana* 3, *P. sitchensis* 1, *P. jezoensis* 1; *Pinus ponderosa* 2, *P. sylvestris* 2, *Pinus* sp. 2; *Pseudotsuga taxifolia* 3; *Tsuga heterophylla* 7; *Betula occidentalis* 1, *B. papyrifera* 2, *Betula* sp. 2; *Populus balsamifera* 2, *P. tremuloides* 3; *Prunus serotina* 1; *Salix* sp. 1. These specimens are from the following localities, in Canada: British Columbia 9, Alberta 2, Saskatchewan 2, Manitoba 4, Ontario 1, New Brunswick 1; in the United States: Arizona 1, California 1, Idaho 1, Minnesota 3, South Dakota 1, Washington 9, Wisconsin 1; in Alaska 2; in Europe: France 1, Sweden 1, Germany 3; and in Japan 2.

Sporophores were obtained from cultures grown on prune agar or on small blocks of coniferous or deciduous wood which had been surface sterilized in acetic acid fumes (3), then placed on the slanted surface of prune agar in 250-cc. flasks. Basidiospores were collected on a sterile cover-slip placed beneath a fruit-body. Spore dilutions were made in sterile distilled water and poured over the surface of lactose gelatine in Petri plates. After germination, isolations were made by cutting out, with a fine needle under the compound microscope, a square of gelatine containing a single spore and placing it in a tube of malt agar. Numbers of single spore isolations were made from each of the cultures listed.

Paired Monosporous Mycelia

(A) From the Same Fruit-body

A series of pairings in all possible combinations of a number of haploid mycelia was made for 20 of the cultures listed in Table I. In every case the haploid mycelia could be divided into two groups. Clamp-connections were formed in every pairing of a member of one group with a member of the other group. The fungi from which the single spore cultures were made are, therefore, "heterothallic" and bipolar. These results corroborate those published earlier, where tables and a detailed explanation are given (4, pp. 41, 45).

It was noted (4, p. 43) that chlamydospores are produced in some cultures of *F. pinicola*. When isolated they germinate readily, and as was to be expected, produce a mycelium which is indistinguishable from the parent mycelium. When chlamydospores from a monosporous culture are isolated and grown, and the mycelia used in pairings, the results are identical in each case with those obtained when the parent mycelium is used.

Monosporous mycelia which had been kept in culture ten years remained in the haploid condition, and their reactions in pairings remained unchanged.

TABLE I

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
283	*Picea mariana*	Timagami, Ont.	285A	*Picea mariana*	Timagami, Ont.	25	25	0
			285B	*Picea mariana*	Timagami, Ont.	25	25	0
			562C	*Tsuga heterophylla*	Vancouver, B.C.	25	0	25
			586	—	Brianion, France	100	100	0
			694	*Pinus* sp.	York Co., N.B.	4	4	0
			831A	*Betula papyrifera*	Timagami, Ont.	29	29	0
			928	*Betula* sp.	Stockholm, Sweden	30	26	4
			5770	*Picea* sp.	Guelph, Ont.	25	25	0
			5778	*Prunus serotina*	York Mills, Ont.	25	25	0
285A	*Picea mariana*	Timagami, Ont.	285B	*Picea mariana*	Timagami, Ont.	98	98	0
			562C	*Tsuga heterophylla*	Vancouver, B.C.	25	0	25
			586	—	Brianion, France	25	24	1
			694	*Pinus* sp.	York Co., N.B.	4	4	0
			831A	*Betula papyrifera*	Timagami, Ont.	28	28	0
			928	*Betula* sp.	Stockholm, Sweden	40	40	0
			1002	?*Pinus* sp.	Madison, Wis.	8	8	0
			1005	*Pinus sylvestris*	Stendal, Germany	8	8	0
			1006	*Salix* sp.	Eberswalde, Germany	9	9	0
			1008	*Pinus sylvestris*	Hirschberg, Silesia	10	10	0
			1015A	?*Tsuga heterophylla*	Vancouver, B.C.	10	10	0
			1025	conifer	Grouse Mt., B.C.	10	0	10
			1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	10	10	0
			1150	*Pseudotsuga taxifolia*	Colville, Wash.	10	10	0
			1151	*Abies grandis*	Lead Point, Wash.	10	10	0
			1152A	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1152B	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	10	10	0
			1155A	*Abies grandis*	Northport, Wash.	8	8	0
			1155B	*Abies grandis*	Northport, Wash.	8	8	0
			1162	*Pinus ponderosa*	Northport, Wash.	8	8	0

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TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections Present	Clamp-connections Absent
285A (*Con.*)	*Picea mariana*	Timagami, Ont.	1169B	*Tsuga heterophylla*	Trail, B.C.	6	0	6
			1258	*Tsuga heterophylla*	Englewood, B.C.	8	0	8
			1259	*Tsuga heterophylla*	Englewood, B.C.	8	8	0
			1264	*Tsuga heterophylla*	Englewood, B.C.	8	8	0
			1265	*Tsuga heterophylla*	Englewood, B.C.	8	8	0
			1268	—	Minaki, Ont.	10	0	10
			1339	—	Japan	10	10	0
			2250	*?Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ? balsamifera*	Victoria Beach, Man.	10	0	10
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	0	10
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	10	0
			3248	conifer	Victoria Beach, Man.	10	10	0
			3249	*Populus balsamifera*	Victoria Beach, Man.	10	0	10
			5563	*Picea glauca*	Edmonton, Alta.	10	10	0
			5564	*Picea glauca*	Edmonton, Alta.	10	10	0
			5770	*Picea* sp.	Guelph, Ont.	25	25	0
			5778	*Prunus serotina*	York Mills, Ont.	25	25	0
			6612	*Pinus ponderosa*	South Dakota	10	10	0
			6613	*Picea* sp.	Arizona	10	0	10
			6616	*Populus tremuloides*	Cloquet, Minn.	10	10	0
			6617	*Populus tremuloides*	Cloquet, Minn.	10	10	0
			6618	*Betula papyrifera*	Cloquet, Minn.	10	6	4
			6624	*Abies concolor*	Susanville, Calif.	10	10	0
			6895	*Picea jezoensis*	Hokkaidô, Japan	10	10	0
			6923	*Picea sitchensis*	Juneau, Alaska	10	0	10
			6925	*Picea glauca*	Hot Springs, Alaska	10	10	0
285B	*Picea mariana*	Timagami, Ont.	562C	*Tsuga heterophylla*	Vancouver, B.C.	25	0	25
			586	—	Brianion, France	25	25	0
			694	*Pinus* sp.	York Co., N.B.	4	4	0
			831A	*Betula papyrifera*	Timagami, Ont.	23	23	0

Continued on page 358

TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
285B (*Con.*)	*Picea mariana*	Timagami, Ont.	928	*Betula* sp.	Stockholm, Sweden	25	25	0
			5770	*Picea* sp.	Guelph, Ont.	25	25	0
			5778	*Prunus serotina*	York Mills, Ont.	25	25	0
526C	*Tsuga heterophylla*	Vancouver, B.C.	586	—	Brianion, France	25	7	18
			694	*Pinus* sp.	York Co., N.B.	4	0	4
			831A	*Betula papyrifera*	Timagami, Ont.	25	0	25
			928	*Betula* sp.	Stockholm, Sweden	40	1	39
			1002	?*Pinus* sp.	Madison, Wis.	8	0	8
			1005	*Pinus sylvestris*	Stendal, Germany	8	1	7
			1006	*Salix* sp.	Eberswalde, Germany	10	0	10
			1008	*Pinus sylvestris*	Hirschberg, Silesia	10	0	10
			1015A	?*Tsuga heterophylla*	Vancouver, B.C.	10	0	10
			1025	conifer	Grouse Mt., B.C.	10	10	0
			1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	10	0	10
			1150	*Pseudotsuga taxifolia*	Colville, Wash.	10	0	10
			1151	*Abies grandis*	Lead Point, Wash.	10	0	10
			1152A	*Picea Engelmanni*	Lead Point, Wash.	8	0	8
			1152B	*Picea Engelmanni*	Lead Point, Wash.	8	0	8
			1153B	*Pseudotsuga* taxifolia	Lead Point, Wash.	10	0	10
			1155A	*Abies grandis*	Northport, Wash.	8	0	8
			1155B	*Abies grandis*	Northport, Wash.	8	0	8
			1162	*Pinus ponderosa*	Northport, Wash.	8	0	8
			1169B	*Tsuga heterophylla*	Trail, B.C.	8	0	8
			1258	*Tsuga heterophylla*	Englewood, B.C.	10	10	0
			1259	*Tsuga heterophylla*	Englewood, B.C.	8	0	8
			1264	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1265	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1268	—	Minaki, Ont.	10	10	0
			1339	—	Japan	10	2	8
			2250	?*Abies balsamea*	Victoria Beach, Man.	10	0	10

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TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
526C (*Con.*)	*Tsuga heterophylla*	Vancouver, B.C.	2251	*Populus ? balsamifera*	Victoria Beach, Man.	10	10	0
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	10	0
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	0	10
			3248	conifer	Victoria Beach, Man.	10	0	10
			3249	*Populus balsamifera*	Victoria Beach, Man.	10	10	0
			5563	*Picea glauca*	Edmonton, Alta.	10	0	10
			5564	*Picea glauca*	Edmonton, Alta.	10	0	10
			5770	*Picea* sp.	Guelph, Ont.	25	1	24
			5778	*Prunus serotina*	York Mills, Ont.	25	0	25
			6612	*Pinus ponderosa*	South Dakota	10	0	10
			6613	*Picea* sp.	Arizona	10	10	0
			6616	*Populus tremuloides*	Cloquet, Minn.	10	0	10
			6617	*Populus tremuloides*	Cloquet, Minn.	10	0	10
			6618	*Betula papyrifera*	Cloquet, Minn.	10	0	10
			6624	*Abies concolor*	Susanville, Calif.	10	6	4
			6895	*Picea jesoensis*	Hokkaidô, Japan	10	3	7
			6923	*Picea sitchensis*	Juneau, Alaska	10	10	0
			6925	*Picea glauca*	Hot Springs, Alaska	10	0	10
586	—	Brianion, France	694	*Pinus* sp.	York Co., N.B.	4	4	0
			831A	*Betula papyrifera*	Timagami, Ont.	25	25	0
			928	*Betula* sp.	Stockholm, Sweden	40	40	0
			1002	*?Pinus* sp.	Madison, Wis.	8	8	0
			1005	*Pinus sylvestris*	Stendal, Germany	8	8	0
			1006	*Salix* sp.	Eberswalde, Germany	10	10	0
			1008	*Pinus sylvestris*	Hirschberg, Silesia	10	10	0
			1015A	*?Tsuga heterophylla*	Vancouver, B.C.	10	7	3
			1025	conifer	Grouse Mt., B.C.	10	0	10
			1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	10	10	0
			1150	*Pseudotsuga taxifolia*	Colville, Wash.	10	10	0

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TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
586 (*Con.*)	—	Brianion, France	1151	*Abies grandis*	Lead Point, Wash.	10	10	0
			1152A	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1152B	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1153B	*Pseudotsuga* taxifolia	Lead Point, Wash.	10	10	0
			1155A	*Abies grandis*	Northport, Wash.	8	8	0
			1162	*Pinus ponderosa*	Northport, Wash.	8	7	1
			1169B	*Tsuga heterophylla*	Trail, B.C.	8	8	0
			1258	*Tsuga heterophylla*	Englewood, B.C.	10	3	7
			1264	*Tsuga heterophylla*	Englewood, B.C.	10	10	0
			1268	—	Minaki, Ont.	10	4	6
			1339	—	*Japan*	10	10	0
			2250	?*Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	5	5
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	10	0
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	10	0
			3248	conifer	Victoria Beach, Man.	10	8	2
			5563	*Picea glauca*	Edmonton, Alta.	10	10	0
			5564	*Picea glauca*	Edmonton, Alta.	10	10	0
			5770	*Picea sp.*	Guelph, Ont.	25	25	0
			5778	*Prunus serotina*	York Mills, Ont.	25	25	0
			6612	*Pinus ponderosa*	South Dakota	10	10	0
			6613	*Picea* sp.	Arizona	10	6	4
			6616	*Populus tremuloides*	Cloquet, Minn.	10	10	0
			6618	*Betula papyrifera*	Cloquet, Minn.	10	10	0
			6624	*Abies concolor*	Susanville, Calif.	10	10	0
			6895	*Picea jezoensis*	Hokkaidô, Japan	10	10	0
			6923	*Picea sitchensis*	Juneau, Alaska	10	0	10
			6925	*Picea glauca*	Hot Springs, Alaska	10	10	0
694	*Pinus* sp.	York Co., N.B.	831A	*Betula papyrifera*	Timagami, Ont.	4	4	0
			928	*Betula* sp.	Stockholm, Sweden	14	14	0

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TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections Present	Clamp-connections Absent
694 (*Con.*)	*Pinus* sp.	York Co., N.B.	1002	?*Pinus* sp.	Madison, Wis.	4	4	0
			1005	*Pinus sylvestris*	Stendal, Germany	8	8	0
			1008	*Pinus sylvestris*	Hirschberg, Silesia	10	10	0
			1025	conifer	Grouse Mt., B.C.	10	0	10
			1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	4	4	0
			1150	*Pseudotsuga taxifolia*	Colville, Wash.	10	10	0
			1151	*Abies grandis*	Lead Point, Wash.	10	10	0
			1152A	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1152B	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	10	10	0
			1155A	*Abies grandis*	Northport, Wash.	8	8	0
			1155B	*Abies grandis*	Northport, Wash.	8	8	0
			1162	*Pinus ponderosa*	Northport, Wash.	8	8	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	8	8	0
			1268	—	Minaki, Ont.	4	0	4
			2251	*Populus* ? *balsamifera*	Victoria Beach, Man.	5	0	5
			5770	*Picea* sp.	Guelph, Ont.	4	4	0
			5778	*Prunus serotina*	York Mills, Ont.	3	3	0
831A	*Betula papyrifera*	Timagami, Ont.	928	*Betula* sp.	Stockholm, Sweden	25	25	0
			1006	*Salix*, sp.	Eberswalde, Germany	9	9	0
			2250	?*Abies balsamea*	Victoria Beach, Man.	10	7	3
			5770	*Picea* sp.	Guelph, Ont.	25	25	0
			5778	*Prunus serotina*	York Mills, Ont.	25	25	0
928	*Betula* sp.	Stockholm, Sweden	1002	?*Pinus* sp.	Madison, Wis.	8	8	0
			1005	*Pinus sylvestris*	Stendal, Germany	8	8	0
			1006	*Salix* sp.	Eberswalde, Germany	10	10	0
			1008	*Pinus sylvestris*	Hirschberg, Silesia	10	10	0
			1015A	?*Tsuga heterophylla*	Vancouver, B.C.	10	10	0
			1025	conifer	Grouse Mt., B.C.	10	3	7

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TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
928 (*Con.*)	*Betula* sp.	Stockholm, Sweden	1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	10	10	0
			1150	*Pseudotsuga taxifolia*	Colville, Wash.	10	10	0
			1151	*Abies grandis*	Lead Point, Wash.	10	10	0
			1152A	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1152B	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	10	10	0
			1155A	*Abies grandis*	Northport, Wash.	8	8	0
			1155B	*Abies grandis*	Northport, Wash.	8	8	0
			1162	*Pinus ponderosa*	Northport, Wash.	8	8	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	8	8	0
			1258	*Tsuga heterophylla*	Englewood, B.C.	10	5	5
			1259	*Tsuga heterophylla*	Englewood, B.C.	10	10	0
			1264	*Tsuga heterophylla*	Englewood, B.C.	10	7	3
			1265	*Tsuga heterophylla*	Engelwood, B.C.	9	5	4
			1268	—	*Minaki, Ont.*	10	2	8
			1339	—	Japan	10	10	0
			2250	*?Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	5	0	5
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	5	5
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	10	0
			3248	conifer	Victoria Beach, Man.	10	0	10
			5563	*Picea glauca*	Edmonton, Alta.	10	10	0
			5564	*Picea glauca*	Edmonton, Alta.	10	10	0
			5770	*Picea* sp.	Guelph, Ont.	25	25	0
			5778	*Prunus serotina*	York Mills, Ont.	40	40	0
			6612	*Pinus ponderosa*	South Dakota	10	10	0
			6613	*Picea* sp.	Arizona	10	8	2
			6616	*Populus tremuloides*	Cloquet, Minn.	10	10	0
			6618	*Betula papyrifera*	Cloquet, Minn.	10	10	0
			6624	*Abies concolor*	Susanville, Calif.	10	10	0
			6895	*Picea jezoensis*	Hokkaidô, Japan	10	10	0

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TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
928 (*Con.*)	*Betula* sp.	Stockholm, Sweden	6923	*Picea sitchensis*	Juneau, Alaska	10	4	6
			6925	*Picea glauca*	Hot Springs, Alaska	10	10	0
1002	?*Pinus* sp.	Madison, Wis.	1005	*Pinus sylvestris*	Stendal, Germany	8	8	0
			1006	*Salix* sp.	Eberswalde, Germany	9	9	0
			1008	*Pinus sylvestris*	Hirschberg, Silesia	8	8	0
			1025	conifer	Grouse Mt., B.C.	10	0	10
			1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	6	4	*2
			1150	*Pseudotsuga taxifolia*	Colville, Wash.	6	6	0
			1152A	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1152B	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	8	8	0
			1155A	*Abies grandis*	Northport, Wash.	8	8	0
			1155B	*Abies grandis*	Northport, Wash.	8	8	0
			1162	*Pinus ponderosa*	Northport, Wash.	8	8	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	8	8	0
			1258	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1268	—	Minaki, Ont.	4	0	4
			1339	—	Japan	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	1	9
1005	*Pinus sylvestris*	Stendal, Germany	1006	*Salix* sp.	Eberswalde, Germany	10	10	0
			1008	*Pinus sylvestris*	Hirschberg, Silesia	8	8	0
			1015A	?*Tsuga heterophylla*	Vancouver, B.C.	10	10	0
			1025	conifer	Grouse Mt., B.C.	10	8	2
			1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	8	7	1
			1258	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1264	*Tsuga heterophylla*	Englewood, B.C.	10	10	0
			1268	—	Minaki, Ont.	8	5	3
			1339	—	Japan	10	10	0
			2250	?*Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	10	0

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TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
1006	*Salix* sp.	Eberswalde, Germany	1008	*Pinus sylvestris*	Hirschberg, Silesia	10	10	0
			1015A	—	Vancouver, B.C.	10	4	6
			1025	conifer	Grouse Mt., B.C.	10	0	10
			1258	*Tsuga heterophylla*	Englewood, B.C.	9	0	9
			1264	*Tsuga heterophylla*	Englewood, B.C.	10	10	0
			1268	—	Minaki, Ont.	9	1	8
			1339	—	Japan	10	10	0
			2250	?*Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	1	9
1008	*Pinus sylvestris*	Hirschberg, Silesia	1015A	?*Tsuga heterophylla*	Vancouver, B.C.	10	9	1
			1025	conifer	Grouse Mt., B.C.	10	2	8
			1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	10	10	0
			1150	*Pseudotsuga taxifolia*	Colville, Wash.	15	15	0
			1152A	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1152B	*Picea Engelmanni*	Lead Point, Wash.	8	8	0
			1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	15	15	0
			1155A	*Abies grandis*	Northport, Wash.	8	8	0
			1155B	*Abies grandis*	Northport, Wash.	8	8	0
			1162	*Pinus ponderosa*	Northport, Wash.	8	8	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	8	8	0
			1258	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1264	*Tsuga heterophylla*	Englewood, B.C.	10	10	0
			1268	—	Minaki, Ont.	10	6	4
			1339	—	Japan	8	8	0
			2250	?*Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	8	2
			5778	*Prunus serotina*	York Mills, Ont.	10	10	0
1015A	?*Tsuga heterophylla*	Vancouver, B.C.	1015B	?*Tsuga heterophylla*	Vancouver, B.C.	15	5	10
			1025	conifer	Grouse Mt., B.C.	10	0	10

Continued on page 365

TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
1015A (Con.)	*?Tsuga heterophylla*	Vancouver, B.C.	1258	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1259	*Tsuga herophylla*	Englewood, B.C.	9	9	0
			1264	*Tsuga heterophylla*	Englewood, B.C.	10	9	1
			1265	*Tsuga heterophylla*	Englewood, B.C.	9	8	1
			1268	—	Minaki, Ont.	10	0	10
			1339	—	Japan	10	10	0
			2250	*?Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	0	10
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	0	10
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	10	0
			3248	conifer	Victoria Beach, Man.	10	10	0
			5563	*Picea glauca*	Edmonton, Alta.	10	9	1
			5564	*Picea glauca*	Edmonton, Alta.	10	10	0
			6612	*Pinus ponderosa*	South Dakota	10	10	0
			6613	*Picea* sp.	Arizona	10	0	10
			6616	*Populus tremuloides*	Cloquet, Minn.	10	10	0
			6618	*Betula papyrifera*	Cloquet, Minn.	10	10	0
			6624	*Abies concolor*	Susanville, Calif.	10	10	0
			6895	*Picea jezoensis*	Hokkaidô, Japan	10	10	0
			6923	*Picea sitchensis*	Juneau, Alaska	10	0	10
			6925	*Picea glauca*	Hot Springs, Alaska	10	10	0
1025	conifer	Grouse Mt., B.C.	1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	6	0	6
			1150	*Pseudotsuga taxifolia*	Colville, Wash.	6	0	6
			1152A	*Picea Engelmanni*	Lead Point, Wash.	6	0	6
			1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	10	0	10
			1155A	*Abies grandis*	Northport, Wash.	6	0	6
			1162	*Pinus ponderosa*	Northport, Wash.	6	0	6
			1169B	*Tsuga heterophylla*	Trail, B.C.	3	0	3
			1258	*Tsuga heterophylla*	Englewood, B.C.	10	8	2
			1259	*Tsuga heterophylla*	Englewood, B.C.	10	0	10

Continued on page 366

TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections Present	Clamp-connections Absent
1025 (*Con.*)	conifer	Grouse Mt., B.C.	1264	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1268	—	Minaki, Ont.	10	10	0
			1339	—	Japan	10	0	10
			2250	*?Abies balsamea*	Victoria Beach, Man.	10	0	10
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	10	0
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	10	0
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	0	10
			3248	conifer	Victoria Beach, Man.	10	0	10
			5563	*Picea glauca*	Edmonton, Alta.	10	0	10
			5564	*Picea glauca*	Edmonton, Alta.	10	0	10
			6612	*Pinus ponderosa*	South Dakota	10	0	10
			6613	*Picea* sp.	Arizona	10	4	6
			6616	*Populus tremuloides*	Cloquet, Minn.	10	0	10
			6618	*Betula papyrifera*	Cloquet, Minn.	10	0	10
			6624	*Abies concolor*	Susanville, Calif.	10	4	6
			6895	*Picea jezoensis*	Hokkaidô, Japan	10	6	4
			6923	*Picea sitchensis*	Juneau, Alaska	10	4	6
			6925	*Picea glauca*	Hot Springs, Alaska	10	0	10
1105	*Betula occidentalis*	Northport, Wash.	2250	*?Abies balsamea*	Victoria Beach, Man.	10	10	0
1120	*Pseudotsuga taxifolia*	Priest Lake, Idaho	1150	*Pseudotsuga taxifolia*	Colville, Wash.	10	10	0
			1152B	*Picea Engelmanni*	Lead Point, Wash.	4	4	0
			1153B	*Picea Engelmanni*	Lead Point, Wash.	10	10	0
			1155B	*Abies grandis*	Northport, Wash.	4	4	0
			1162	*Pinus ponderosa*	Northport, Wash.	6	6	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	4	4	0
			1268	—	Minaki, Ont.	4	0	4
			5778	*Prunus serotina*	York Mills, Ont.	10	10	0

Continued on page 367

TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
1150	*Pseudotsuga taxifolia*	Colville, Wash.	1152B	*Picea Engelmanni*	Lead Point, Wash.	4	4	0
			1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	15	15	0
			1155B	*Abies grandis*	Northport, Wash.	4	4	0
			1162	*Pinus ponderosa*	Northport, Wash.	6	6	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	4	4	0
			1268	—	Minaki, Ont.	10	0	10
			2251	*Populus balsamifera*	Victoria Beach, Man.	10	0	10
			5778	*Prunus serotina*	York Mills, Ont.	10	10	0
1151	*Abies grandis*	Lead Point, Wash.	1152B	*Picea Engelmanni*	Lead Point, Wash.	4	4	0
			1155B	*Abies grandis*	Northport, Wash.	4	4	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	4	4	0
			5778	*Prunus serotina*	York Mills, Ont.	8	8	0
1152A	*Picea Engelmanni*	Lead Point, Wash.	1152B	*Picea Engelmanni*	Lead Point, Wash.	30	17	13
			1162	*Pinus ponderosa*	Northport, Wash.	6	6	0
			1268	—	Minaki, Ont.	8	0	8
1152B	*Picea Engelmanni*	Lead Point, Wash.	1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	8	8	0
			1155B	*Abies grandis*	Northport, Wash.	4	4	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	4	4	0
			1268	—	Minaki, Ont.	8	0	8
1153B	*Pseudotsuga taxifolia*	Lead Point, Wash.	1155A	*Abies grandis*	Northport, Wash.	8	8	0
			1155B	*Abies grandis*	Northport, Wash.	8	8	0
			1162	*Pinus ponderosa*	Northport, Wash.	8	8	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	8	8	0
			1268	—	Minaki, Ont.	10	0	10
			5778	*Prunus serotina*	York Mills, Ont.	10	10	0

Continued on page 368

TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
1155A	*Abies grandis*	Northport, Wash.	1155B	*Abies grandis*	Northport, Wash.	70	38	32
			1162	*Pinus ponderosa*	Northport, Wash.	6	6	0
			1169B	*Tsuga heterophylla*	Trail, B.C.	6	6	0
			1268	—	Minaki, Ont.	8	0	8
			2250	?*Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ? balsamifera*	Victoria Beach, Man.	10	0	10
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	1	9
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	10	0
			3248	conifer	Victoria Beach, Man.	5	5	0
			5563	*Picea glauca*	Edmonton, Alta.	10	10	0
			5564	*Picea glauca*	Edmonton, Alta.	10	10	0
			6612	*Pinus ponderosa*	South Dakota	10	8	2
			6613	*Picea* sp.	Arizona	10	5	5
			6616	*Populus tremuloides*	Cloquet, Minn.	10	10	0
			6618	*Betula papyrifera*	Cloquet, Minn.	10	10	0
			6624	*Abies concolor*	Susanville, Calif.	10	10	0
			6895	*Picea jezoensis*	Hokkaidô, Japan	10	10	0
			6923	*Picea sitchensis*	Juneau, Alaska	10	0	10
			6925	*Picea glauca*	Hot Springs, Alaska	10	10	0
1155B	*Abies grandis*	Northport, Wash.	1268	—	Minaki, Ont.	8	0	8
			3248	conifer	Victoria Beach, Man.	5	5	0
1162	*Pinus ponderosa*	Northport, Wash.	1169B	*Tsuga heterophylla*	Trail, B.C.	3	3	0
			1268	—	Minaki, Ont.	8	0	8
1169B	*Tsuga heterophylla*	Trail, B.C.	1169C	*Tsuga heterophylla*	Trail, B.C.	8	8	0
			1268	—	Minaki, Ont.	8	0	8
			1339	—	Japan	10	10	0
			2250	?*Abies balsamea*	Victoria Beach, Man.	9	9	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	1	9

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TABLE I—*Continued*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Continued*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
1258	*Tsuga heterophylla*	Englewood, B.C.	1259	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1264	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1265	*Tsuga heterophylla*	Englewood, B.C.	10	0	10
			1268	—	Minaki, Ont.	10	10	0
			1339	—	Japan	16	6	10
			2250	*?Abies balsamea*	Victoria Beach, Man.	9	0	9
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	9	9	0
1259	*Tsuga heterophylla*	Englewood, B.C.	1264	*Tsuga heterophylla*	Englewood, B.C.	10	10	0
			1265	*Tsuga heterophylla*	Englewood, B.C.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	0	10
1264	*Tsuga heterophylla*	Englewood, B.C.	1265	*Tsuga heterophylla*	Englewood, B.C.	10	5	5
			1268	—	Minaki, Ont.	10	2	8
			1339	—	Japan	10	10	0
			2250	*?Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	2	8
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	0	10
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	10	0
			3248	conifer	Victoria Beach, Man.	10	10	0
			5563	*Picea glauca*	Edmonton, Alta.	10	10	0
			5564	*Picea glauca*	Edmonton, Alta.	10	10	0
			6612	*Pinus ponderosa*	South Dakota	10	10	0
			6613	*Picea* sp.	Arizona	6	1	5
			6616	*Populus tremuloides*	Cloquet, Minn.	10	10	0
			6617	*Populus tremuloides*	Cloquet, Minn.	6	6	0
			6618	*Betula papyrifera*	Cloquet, Minn.	10	10	0
			6624	*Abies concolor*	Susanville, Calif.	10	10	0
			6895	*Picea jesoensis*	Hokkaidô, Japan	10	10	0
			6923	*Picea sitchensis*	Juneau, Alaska	10	0	10
			6925	*Picea glauca*	Hot Springs, Alaska	10	10	0

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TABLE I—*Concluded*

Fomes pinicola. DETAILED LIST OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES—*Concluded*

Culture number	Host	Locality	Paired with culture number	Host	Locality	Number of pairings	Clamp-connections	
							Present	Absent
1265	*Tsuga heterophylla*	Englewood, B.C.	2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	1	9
1268	—	Minaki, Ont.	1339	—	Japan	10	2	8
			2250	*?Abies balsamea*	Victoria Beach, Man.	10	0	10
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	10	0
			5778	*Prunus serotina*	York Mills, Ont.	10	0	10
1339	—	Japan	2250	*?Abies balsamea*	Victoria Beach, Man.	10	10	0
			2251	*Populus ?balsamifera*	Victoria Beach, Man.	10	8	2
			2379	*Populus tremuloides*	Lake Waskesiu, Sask.	10	6	4
			2380	*Picea* sp.	Lake Waskesiu, Sask.	10	10	0
			3248	conifer	Victoria Beach, Man.	10	10	0
			5563	*Picea glauca*	Edmonton, Alta	10	8	2
			5564	*Picea glauca*	Edmonton, Alta.	10	10	0
			6612	*Pinus ponderosa*	South Dakota	10	10	0
			6613	*Picea* sp.	Arizona	10	6	4
			6616	*Populus tremuloides*	Cloquet, Minn.	10	10	0
			6618	*Betula papyrifera*	Cloquet, Minn.	10	10	0
			6624	*Abies concolor*	Susanville, Calif.	10	10	0
			6895	*Picea jezoensis*	Hokkaidô, Japan	10	10	0
			6923	*Picea sitchensis*	Juneau, Alaska	10	0	10
			6925	*Picea glauca*	Hot Springs, Alaska	10	10	0
2250	*?Abies balsamea*	Victoria Beach, Man.	2251	*Populus ?balsamifera*	Victoria Beach, Man.	32	4	28
5770	*Picea* sp.	Guelph, Ont.	5778	*Prunus serotina*	York Mills, Ont.	25	25	0

(B) From Different Fruit-bodies

In Table I a detailed list is given of the pairings which have been made, using monosporous mycelia of *F. pinicola* from different sources. It includes the culture number, host, and locality of each isolate used, the total number of pairings made, and the numbers in which clamp-connections were present or absent. In the earlier part of the investigation a new isolate was paired with most or all of the other isolates for which monosporous mycelia were available. Later, when the existence of three groups seemed fairly definite,

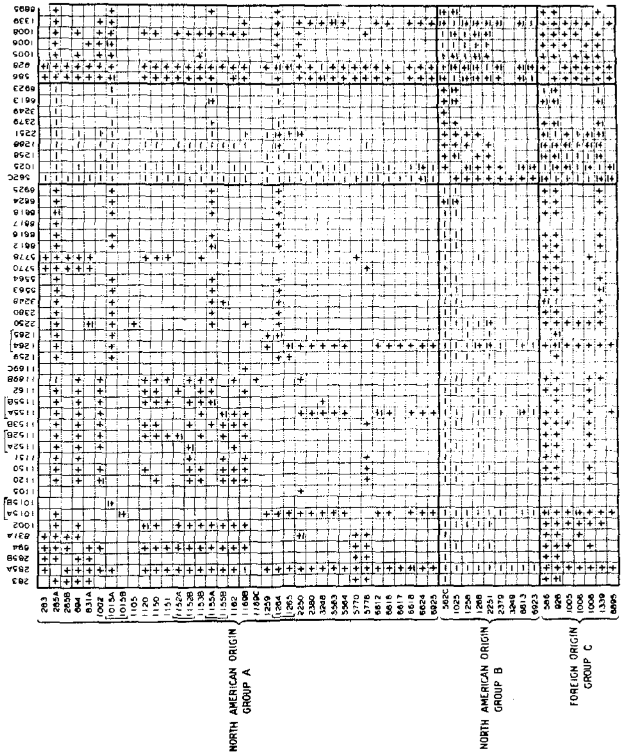

TABLE II. *The results of pairings of monosporous mycelia of Fomes pinicola from various hosts and localities.*

a new isolate was paired with only a few representatives of each group. The results of such pairings established the group to which the new isolate belonged.

In Table II these results are summarized and presented graphically. The following signs have been used:

(+) to indicate that clamp-connections were produced in every pairing of a monosporous mycelium from one source with one from the second source.

(−) to indicate that no clamp-connections were found in any pairing of monosporous mycelia from these two sources.

(±) to indicate the presence of clamp-connections in the greater proportion of pairings made, but not in all.

(∓) to indicate the absence of clamp-connections in the greater proportion of pairings made.

(+.) to indicate that *with one exception* clamp-connections were produced in every pairing made.

(−.) to indicate that *with one exception* no clamp-connections were produced in any pairing.

From Table II it is evident that in general the isolates of North American origin may be divided into two groups, a large Group A, and a small Group B. Monosporous mycelia from an isolate in Group A are compatible (mutually fertile) with monosporous mycelia from other members of Group A; similarly monosporous mycelia from an isolate in Group B are compatible with other monosporous mycelia of Group B; but monosporous mycelia from isolates in Group A are almost completely incompatible with monosporous mycelia from Group B. This might suggest the possibility of the presence of two species were it not for the results obtained when monosporous mycelia from Group A and from Group B are paired with those from a number of isolates of non-American origin. The latter form a third Group C, the members of which are completely compatible among themselves, almost completely compatible with Group A, and *only partially incompatible with Group B.*

The geographical distribution of these three, Group A (●), Group B (○), and Group C (⊙), is shown in Fig. 1. Group A, which contains isolates from species of *Abies*, *Picea*, *Pinus*, *Pseudotsuga*, *Tsuga*, *Betula*, *Populus*, and *Prunus*, has a known geographical range in Canada of British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, and New Brunswick, in the United States a range of California, Washington, Idaho, South Dakota, Minnesota and Wisconsin, and in Alaska; Group B, which contains isolates from *Picea*, *Tsuga*, and *Populus*, has a known geographical range of British Columbia, Saskatchewan, Manitoba, and Ontario in Canada, Arizona in the United States, and Alaska; Group C, which contains isolates from species of *Picea*, *Pinus*, *Betula*, and *Salix*, has a known geographical range of France, Sweden, Germany and Japan.

Culture 1002 was of interest because it was received from the Centraalbureau voor Schimmelcultures, Baarn, Holland, and was presumed to be of European

origin (Group C). However, when pairings were made, the results were identical with those found in Group A of North American material. Later, Dr. Westerdijk assured us that the culture came originally from Wisconsin.

As only nine of a total of 52 isolates tested were found to belong to Group B, the proportion of such incompatible isolates would seem to be small. The nine isolates came from Vancouver and Englewood in British Columbia, Lake Waskesiu in Saskatchewan, Victoria Beach in Manitoba, Minaki in western Ontario, Arizona and Alaska. With the exception of Arizona, from which only the one culture was available, members of Group A have also been found in these same localities. The isolates in Group B came from *Tsuga*, *Picea*, and *Populus*, and members of Group A have been found on these same host species. Since members of Group A have been found on each host species on which a member of Group B occurred, and in each locality, it seems definite that incompatibility in *Fomes pinicola* is not influenced primarily either by host or geographical distribution.

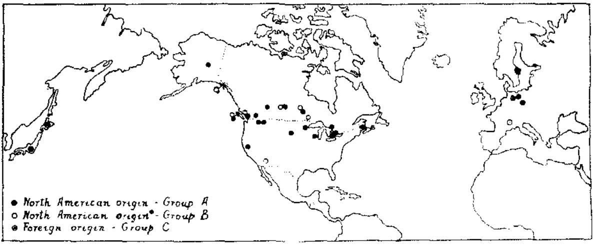

FIG. 1. *Map showing the geographical distribution of the collections of Fomes pinicola from which monosporous isolations were obtained and pairings made.*

Members of Group B are of further interest because they include three cultures, 2251, 2379, and 3249, isolated from sporophores which grew on *Populus* and typical of the form common on that host—a form which is sufficiently distinct that at times it has been considered a variety or has been given specific rank as *F. marginatus*. Such sporophores are a more or less uniform white, buff, or light gray with occasionally a darker band, but with no trace of the red coloration or resinous exudate so characteristic of *F. pinicola*. The remainder of the isolates in Group B, however, although they behave in identical fashion, originated from fruit-bodies of the type commonly found on coniferous hosts, fruit-bodies which were indistinguishable macroscopically or microscopically from sporophores used as sources of cultures in Group A. Interestingly enough, members of compatible and incompatible groups (A and B) were found side by side in British Columbia at Englewood and in the vicinity of Vancouver, in Saskatchewan at Lake Waskesiu, in Manitoba at

Victoria Beach, and both occur in Alaska (Fig. 1). They are separable only after pairings have been made. Conversely, not all isolates from *Populus* fall into Group B; 6616 and 6617 both grew on *Populus tremuloides* at Cloquet, Minn., yet pairings show that they belong in Group A.

Through the courtesy of Miss Dorothy Blaisdell we have just received the following notes on U.S.D.A. specimens 59015 and 59019, from which cultures 6616 and 6617 respectively were made. The former "has a rather wide red margin and is a dark gray to dark mahogany brown", the latter "is a light gray and has a very narrow margin of red". The presence of such a red margin would indicate that though these sporophores grew on *Populus* they do not belong to the typical so-called *Populus* form.

(C) From Two Fruit-bodies Which Grew on the Same Tree

One example was given previously (4, p. 48, Table IX, p. 45) where pairings were made between monosporous mycelia which originated from two different sporophores, 285A and 285B, growing on a specimen of *Picea mariana* at Timagami, Ont. Any monosporous mycelium from 285A was compatible with any mycelium from 285B. Apparently then, there was more than one source of infection in this tree, and the mycelium which produced 285A was of different origin from that which produced 285B. If they had both originated from the same diploid mycelium, and monosporous mycelia from the two fruit-bodies had been paired, they would "react together in every respect as though they had been isolated from a single fruit-body" (2 p. 442).

This example is included in Table II together with five other pairs of fruit-bodies which were tested subsequently:— cultures 1015A and 1015B from two sporophores on (?)*Tsuga heterophylla*, Vancouver, B.C.; 1152A and 1152B from two sporophores on *Picea Engelmanni*, Lead Point, Wash.; 1155A and 1155B, from two sporophores on *Abies grandis*, Northport, Wash.; 1264 and 1265 from two sporophores on *Tsuga heterophylla*, Englewood, B.C.; and 1169B and 1169C from two sporophores on *Tsuga heterophylla*, Trail, B.C. In every case but one, the two isolates were identical when judged by their behavior in pairings, *i.e.*, monosporous mycelia from them reacted as if they had been isolated from a single fruit-body; but monosporous mycelia from 1169B and 1169C were compatible when paired, which indicates, as in the case of 285A and 285B cited above, two separate infections in the one tree.

As was pointed out earlier (4, pp. 44, 48), the behavior of *F. pinicola* does not conform entirely with that reported by Vandendries for *Coprinus micaceus* (5, 6). He made an exhaustive study of the behavior of American and European isolates of this fungus, but only those conclusions based on experiments which have been duplicated more or less with isolates of *F. pinicola* will be considered here. Vandendries (6) found: (1) "Entre souches d'une même région, mais suffisamment distantes pour qu'on puisse les considérer comme étrangères l'une à l'autre, la fertilité est la règle. Cette loi souffre cependant des exceptions, dues à des mutations; (2) Les souches européennes, cueillies à des grandes distances les unes des autres, sont, en général, stériles entre elles.

Il y a pour cette loi des exceptions que les conclusions 4 et 5 expliquent; (3) Les souches américaines sont portées à la fertilité entre elles. On trouve à cette loi des exceptions assez nombreuses entre souches très éloignées l'une de l'autre; (4) Entre races américaines et races européennes, la stérilité est générale. Les rares exceptions à cette loi s'expliquent par des mutations individuelles." His final conclusion was: "A la surface du globe existe actuellement pour *Coprinus micaceus* un état d'équilibre sexuel, dominé par deux conditions: 1°. les populations très éloignées l'une de l'autre sont stériles quand on les met en contact; 2°. les populations d'une région donnée sont fertiles entre elles.

"Toute perturbation dans cet état d'harmonie naturelle trouve sa source dans des mutations. Celles-ci sont indépendantes de facteurs extrinsèques tels que climat, latitude, habitat, agents naturels extérieurs.

"Les individus mutants ne constituant qu'une minorité, l'état d'équilibre défini est appelé à persister, comme conséquence inéluctable des lois de l'hérédité et de la loi du nombre."

A glance at Table II shows that as far as *F. pinicola* is concerned, (i) fertility is the rule; (ii) isolates of European origin, from France, Sweden, and Germany, as well as those from Japan were all mutually fertile (compatible); (iii) American isolates tend to be fertile among themselves; (iv) fertility (compatibility) is the rule, sterility the exception, between European and American isolates.

In his study of sexuality of *Auricularia Auricula-Judae*, Barnett (1) used seven collections which originated in New York, Nebraska, North Carolina, Iowa and Colorado respectively. He found this fungus typically bipolar. Complete compatibility existed between five of the seven collections, while the remainder were partially or completely incompatible. The two isolates involved in these irregular pairings were the ones from New York and Colorado. Barnett (1, p. 645) also notes, "In addition to pairings between fruit-bodies of different collections, complete compatibility was found to exist between two fruit-bodies of *A. Auricula-Judae* taken from the same collection. This was also true of two fruit-bodies of *Exidia saccharina* growing on the same stick within 12 in. of each other, and for two fruit-bodies of *E. glandulosa* growing but 2 in. apart."

The similarity between the behavior of *F. pinicola* and *A. Auricula-Judae* is striking. *F. pinicola* is bipolar. Isolates of North American origin are in the main compatible, though there are a few which are incompatible and a very few which are partially compatible. Two instances have been given of complete compatibility between two fruit-bodies growing on the same tree. In addition, it has been shown for *F. pinicola* *(a)* that among isolates of North American origin those few which are incompatible with the majority of isolates are compatible among themselves, and *(b)* that isolates of non-American origin are completely compatible among themselves and with the majority of isolates of North American origin, and only partially incompatible with the remainder.

Rotting of Wood by Monosporous Mycelia of Fomes pinicola

Large test tubes each containing 17 cc. of malt agar and one block of *Picea sitchensis* 3 by ½ by ½ in. were sterilized for 30 min. at 15 lb. steam pressure. Later they were inoculated with agar from cultures of monosporous mycelia of *Fomes pinicola*, incubated at 25° C. until a mycelium began to grow from the inoculum, then kept on a shelf in the laboratory at room temperature.

Six months later all the cultures had dried out, and were moistened. At the end of seven months the wood blocks were removed from the tubes and examined. The blocks in eight of the tubes showed little change; apparently the fungus had not taken hold before the culture dried. Of the remaining three, the block inoculated with culture No. 928-2 (Sweden) showed typical rot within though no sign of growth on the outside, while those inoculated with 562C-4 (British Columbia) and 694-1 (New Brunswick) were covered with a dense mycelial mat, and the wood was in an advanced stage of decay. In sections of wood rotted by monosporous cultures, hyphae were present and could be traced for some distance, but all showed simple septa instead of the usual clamp-connections.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

I. NITROGEN DISTRIBUTION AMONG PROTEIN FRACTIONS OF BARLEY[1]

By J. Ansel Anderson[2] and C. Alan Ayre[2]

Abstract

Determinations of total nitrogen and nitrogen fractions were made on 144 samples of barley representing 12 varieties grown at each of 12 widely separated experimental stations in Canada.

A highly significant positive correlation between alcohol-soluble protein nitrogen and total nitrogen was found both within and between varieties. No correlation between total nitrogen and other nitrogen fractions was found between varieties; but significant positive correlations were found within varieties, that for insoluble protein nitrogen being considerably higher than those for total salt-soluble nitrogen, salt-soluble protein nitrogen, and non-protein nitrogen. With increasing total nitrogen, the proportion in salt-soluble form decreases, that in alcohol-soluble form increases, and that in insoluble form remains relatively constant. The results thus offer further support for Bishop's "Protein regularity principle".

Mean varietal differences were found with respect to each nitrogen fraction, but elucidation of differences in nitrogen distribution patterns was complicated by the effect of varietal differences in total nitrogen content. Statistical analyses demonstrated the validity of eliminating this effect by adjusting varietal means for fractions to values corresponding to equal total nitrogen contents. When this was done it was found: that the three two-rowed varieties (Charlottetown 80, Hannchen, and Victory) were higher in alcohol-soluble protein nitrogen and lower in insoluble protein nitrogen than any of the six-rowed varieties; and that the four smooth-awned six-rowed varieties (Nobarb, Regal, Velvet, and Wisconsin 38) were lower in total salt-soluble nitrogen and higher in insoluble nitrogen than any of the rough-awned six-rowed varieties (O.A.C. 21, Mensury, Ott. 60, Olli, Peatland, and Pontiac). Owing to the variation between varieties within classes, and the small number of varieties studied, the average differences between the three classes are not statistically significant. Nevertheless, since by comparison with the rough-awned six-rowed varieties, the two-rowed varieties yield higher malt extracts, and the four smooth-awned varieties yield lower malt extracts and are lower in enzymatic activity, the indications of a possible relation between nitrogen distribution and malting quality are interesting.

As a result of investigations made by Bishop (1, 3), Hofman-Bang (7), and Fink and Kunisch (5), it is apparent that the distribution of nitrogen among the various protein fractions of barley is greatly influenced by the environment in which the barley is grown and that the environmental effect on this distribution is closely related to the effect on total nitrogen content. Thus it has been demonstrated that with increasing total nitrogen, within any variety, the percentage of total nitrogen in salt-soluble form decreases, the percentage in alcohol-soluble form increases, and the percentage in insoluble form remains relatively constant. It has also been shown that the distribution of the nitrogen is influenced by variety so that, at any given total nitrogen content, the proportions of each protein fraction present may differ from one variety to the next.

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[2] *Biochemist, National Research Laboratories, Ottawa.*

The authors listed above, and others cited by them, confined their investigations almost entirely to two-rowed varieties, and no similar investigations of the six-rowed Manchurian varieties used for the preparation of brewers' malt on this continent have come to the authors' attention. Moreover, in general, the varieties studied differed little in malting qualities. As a result, no direct relation between nitrogen distribution in different varieties and their malting qualities has yet been established, although it has been inferred that with some varieties, high permanently soluble nitrogen in the wort is the result, at least in part, of high salt-soluble nitrogen in the barley.

The present investigation was undertaken with the object of determining differences in the nitrogen distribution amongst a representative set of the barley varieties grown in Canada. The set includes five rough-awned six-rowed varieties, four smooth-awned six-rowed varieties, and three two-rowed varieties. In order to facilitate investigation of possible relations between nitrogen distribution and other barley and malt properties, the varieties were selected so as to provide a range differing as widely as possible in biochemical and agronomic characteristics. Moreover, all the varieties were grown at each of 12 widely separated experimental stations in Canada, and additional study of the effect of environment on nitrogen distribution was therefore possible.

This paper deals only with data collected in the initial study of the distribution of the nitrogen among the various protein fractions. The relation of these data to those on other barley and malt qualities will be reported in following papers of this series.

Materials

The investigation was made with 12 varieties of barley, each of which was grown in 1937 at 12 experimental stations in Canada.

Varieties

O.A.C. 21 is a six-rowed, rough-awned variety of the Manchurian type with a blue aleurone layer and short-haired rachilla. This variety is very satisfactory for the production of brewers' malts under Canadian conditions, and it is officially recognized as a standard for malting quality, since the Canada Grain Act specifies that all varieties admitted to the top three grades of Canada Western Six-row Barley shall be "equal in value for malting purposes to O.A.C. 21." The variety yields moderately well over a wide range of environments, but the straw is rather weak and the heads tend to break off at the neck. Although O.A.C. 21 cannot be considered entirely satisfactory from the agronomic viewpoint, it is still widely grown in both eastern and western Canada because of its superior malting qualities.

Mensury, Ott. 60 is almost indistinguishable in appearance and properties from O.A.C. 21 and is admitted to the malting grades. It has a somewhat stiffer straw and is rather widely grown in the Province of Manitoba.

Olli is also similar to O.A.C. 21, but has a somewhat smaller kernel. Although it is not yet admitted to the malting grades, it is considered promising

from the malting viewpoint, mainly because of its high extract yield and high enzymatic activity. The variety was introduced from Finland and is one of the earliest maturing of those grown in Canada. For this reason it is becoming popular in the Peace River District in northern Alberta and may spread to other northern areas.

Peatland has a small kernel like Olli but differs from it in that the aleurone layer is yellow. Although admitted to the malting grades, it is now considered of doubtful value for malting purposes because of its tendency to produce grain of high nitrogen content. The variety was introduced in the belief that it would be suitable for the gray wooded soils of north-central Alberta, but it has hardly lived up to its earlier promise. Its stiff straw and resistance to stem rust make it an attractive parent for breeding work, in which it is now being widely exploited.

Pontiac is also a six-rowed, rough-awned variety similar in general characteristics to O.A.C. 21. It is a comparatively new selection from Mensury and has not yet been licensed for general distribution. Comparatively little is known of its malting properties, but data obtained in the laboratory suggest that these are satisfactory.

Nobarb, *Regal*, *Wisconsin Ped. 38*, and *Velvet* are all smooth-awned, six-rowed varieties developed from crosses in which the smooth-awned variety Lion was one of the original parents. Nobarb has a short-haired rachilla and the others have long-haired rachillae. All have yellow aleurone layers. In Canada, these varieties are considered unsatisfactory for malting purposes. The hulls are loosely attached, and as a result the acrospires tend to split the hulls during germination, thus producing a ragged, unevenly grown malt which is subject to further damage during the cleaning process. All four varieties yield malts with comparatively low extract, and all but Velvet are characterized by low enzymatic activity. These smooth-awned varieties are popular with farmers because they are more pleasant to handle and yield straw which is more suitable for feeding purposes. In general, they are somewhat more satisfactory from the agronomic viewpoint than the rough-awned six-rowed varieties. As a result, their popularity is growing steadily amongst farmers in both eastern and western Canada.

Hannchen, *Charlottetown 80*, and *Victory* are rough-awned, two-rowed varieties with yellow aleurone layers and long-haired rachillae. The first has a compact wide head, and the other two have lax narrow heads. The environmental conditions which prevail over most of the barley-growing areas in Canada tend to produce grain of comparatively high nitrogen content. Under these conditions the two-rowed barleys with their larger kernels produce grain which fails to modify adequately in the six-day germination period used in Canada. For this reason, the amount of two-rowed barley malted in Canada is almost negligible, and little is known of the comparative malting qualities of the two-rowed varieties. These, however, are grown fairly widely for feed purposes. Hannchen is confined largely to the Prairie Provinces, whereas Charlottetown 80 is popular in the east. Victory is a

comparatively new introduction from Sweden and shows some promise in Alberta.

Stations

The barleys were grown in 1937 at the following twelve experimental stations:

1. Dominion Experimental Farm, Nappan, Nova Scotia.
2. Dominion Experimental Station, Fredericton, New Brunswick.
3. Dominion Experimental Station, Ste. Anne de la Pocatière, Quebec.
4. Macdonald College, Ste. Anne de Bellevue, Quebec.
5. Ontario Agricultural College, Guelph, Ontario.
6. Central Experimental Farm, Ottawa, Ontario.
7. Dominion Experimental Farm, Brandon, Manitoba.
8. Dominion Illustration Station, Gilbert Plains, Manitoba.
9. University of Manitoba, Winnipeg, Manitoba.
10. Dominion Experimental Sub-station, Beaverlodge, Alberta.
11. Dominion Experimental Station, Lacombe, Alberta.
12. Dominion Experimental Station, Lethbridge, Alberta.

It will be observed that these stations are scattered over the grain-growing areas in Canada from the east coast to the Rocky Mountains. Wide-spread drought in Saskatchewan in 1937 prevented the inclusion of stations in that province.

Field Plan

The 12 varieties studied were selected from a total of 24 which were being grown in a trial conducted annually for the study of the agronomic characteristics of the varieties. The seed used was authentic pedigreed stock distributed from the Central Experimental Farm, Ottawa. The barleys were grown in plots of five rod-rows arranged in a modified balanced block with quadruplicate plots for each variety. Marginal effects were reduced by harvesting only the central three rows of each plot. The samples used in the present study were obtained by bulking and mixing the grain from the four quadruplicate plots and subsequently obtaining a small representative aliquot by the use of a Boerner Sampler.

Laboratory Methods

The samples were ground in a Wiley mill and then, following the advice of Hofman-Bang (6), they were ground for 24 hr. in a ball mill.

Total salt-soluble nitrogen and alcohol-soluble protein nitrogen were determined on the same 5-gm. sample by Bishop's methods (2). Insoluble protein nitrogen was calculated by subtracting the sum of these two fractions from total nitrogen. Non-protein nitrogen was determined, as an afterthought, by extracting a second 5-gm. sample with salt solution according to Bishop's method, precipitating the protein nitrogen with 5% trichloracetic, filtering, and determining the nitrogen in an aliquot of the filtrate. Salt-soluble

protein nitrogen was calculated by subtracting non-protein nitrogen from total salt-soluble nitrogen.

Preliminary investigations showed that results could be reproduced satisfactorily by the methods used. However, in order to provide a check on the precision of analyses during the investigation, duplicate determinations were made on one-third of the samples and standard deviations were calculated from the data thus obtained. The samples on which duplicate determinations were made were selected at random, after imposing the limitation that four samples of each variety and four samples from each station should be chosen. Varietal and station means have therefore equal weights and comprise the results of 16 determinations, *i.e.* single determinations on eight samples and duplicate determinations on four more. The standard deviations of the means of 16 determinations, in terms of nitrogen as percentage of dry matter, were found to be as follows:— non-protein nitrogen, 0.004; total salt-soluble nitrogen, 0.006; and alcohol-soluble protein nitrogen, 0.005.

Total nitrogen was determined in duplicate on all samples. One set of determinations was made on the samples ground in the ball mill and a second set was made on separate aliquots of barley ground in a Wiley mill only. Varietal and station means thus represent the mean of 24 determinations, and an estimate of the combined sampling and analytical errors is available for the determination of total nitrogen. The standard deviation of the mean of 24 determinations proved to be 0.009.

Results and Discussion

Relation Between Total Nitrogen and Nitrogen of Fractions

The data on total nitrogen and on the various fractions into which it was separated are summarized in Table I as means for each station over all varieties. The stations are listed in order of increasing total nitrogen content of the barley and all data are given as percentages of dry matter.

It is apparent that the nitrogen of each fraction tends to increase with increasing total nitrogen content. A measure of the degree of association of total nitrogen with the nitrogen of each fraction is provided by the coefficients of correlation given in the last line but one of the table. These all proved to be highly significant, but there are marked differences between them. It is apparent that the relation between total nitrogen and alcohol-soluble nitrogen is quite close, between total nitrogen and insoluble protein nitrogen is fairly close, and that the relations between total nitrogen and the other fractions are of a much lower order.

A possible explanation of the differences in the degree of association of total nitrogen with the nitrogen of the various fractions may lie in the methods of determining the latter. These methods effect arbitrary separations, particularly when applied to the mixtures of hulls, germs, bran, aleurone, and endosperm which result when barley is ground. Of the various fractions separated, there can be little doubt that the alcohol-soluble portion most nearly represents a single protein. The amount of alcohol-soluble nitrogen

TABLE I

MEAN TOTAL NITROGEN AND NITROGEN FRACTIONS FOR EACH STATION, AS PERCENTAGES OF DRY MATTER

Station	Total nitrogen	Salt-soluble			Alcohol-soluble protein nitrogen	Insoluble protein nitrogen
		Non-protein nitrogen	Protein nitrogen	Total		
Nappan	1.540	.292	.305	.598	.480	.462
Fredericton	1.738	.342	.320	.662	.582	.494
Ste. Anne de Bellevue	1.932	.333	.312	.646	.699	.587
Ste. Anne de la Pocatière	2.278	.338	.348	.686	.893	.699
Lethbridge	2.294	.349	.462	.812	.893	.589
Winnipeg	2.333	.359	.398	.757	.921	.655
Brandon	2.357	.342	.399	.741	.962	.654
Guelph	2.381	.352	.445	.797	.932	.652
Ottawa	2.526	.361	.411	.772	1.030	.724
Lacombe	2.668	.395	.396	.791	1.182	.695
Beaverlodge	2.674	.349	.393	.742	1.208	.723
Gilbert Plains	2.687	.394	.412	.807	1.065	.815
Coefficient of correlation with total nitrogen*		.823	.736	.851	.985	.926
Coefficient of regression on total nitrogen		.060	.104	.167	.589	.249

* *Required for 1% level of significance, 0.708.*

in the hulls and germs is almost negligible (9) and the fraction isolated is probably obtained almost entirely from the remainder of the kernel. According to Osborne (8) and most subsequent investigators, the alcohol-soluble fraction represents a single protein, namely, hordein. On the other hand, Rose and Anderson (9) believe that alcohol removes an arbitrarily chosen fraction of a protein complex. This fraction, however, may well represent a fairly constant proportion of the protein complex.

In contrast, it seems clear that the other fractions represent mixtures of various degrees of complexity. The insoluble portion appears to represent a part of the main protein complex, mixed with smaller amounts of an entirely different protein from the germ (9), and other proteins from the hulls and other parts of the kernel. The salt-soluble fraction consists of mixtures of albumins and globulins obtained from both endosperm and germ, and some non-protein nitrogen also appears to exist in each part of the kernel.

With these considerations in mind, it is perhaps not surprising that the highest degree of association is found between total nitrogen and the most distinct protein fraction, namely, the alcohol-soluble one. Such associations as may exist between total nitrogen and other individual proteins may well be partly masked by admixture of fractions with which total nitrogen is not closely associated.

The functions of the various protein fractions in the metabolism of the plant also have a bearing on this question. According to Bishop (4), the hordein (alcohol-soluble protein) represents a reserve or storage protein.

It is laid down during the filling process and presumably plays no further part in the metabolism until it is again drawn upon when the seed germinates. If this is true, it seems logical to suppose that as increasing amounts of nitrogen are transferred to the grain, increasing amounts will be stored as hordein, and a close relation between total nitrogen and the alcohol-soluble fraction would thus be expected. According to Rose and Anderson (9), the alcohol-soluble fraction and the main portion of the insoluble fraction should be considered as one protein complex, presumably functioning as a reserve. It follows that a close relation between total nitrogen and the insoluble protein fraction is also to be expected, though this degree of association might be lowered by the masking effect of admixtures of small amounts of other fractions from other parts of the grain. The results of the present investigation fit these hypotheses, since it is apparent that a large proportion of the environmental factors which govern the total nitrogen content of the grain also control the amounts of alcohol-soluble protein and insoluble protein laid down in it.

On the other hand, it seems probable that the more soluble proteins and the non-protein nitrogen compounds are functioning in the metabolic processes of the grain at the time of harvesting. The amounts of these present in the grain, though related to the total nitrogen content, may be governed also by prevailing, or recently prevailing, environmental conditions which have comparatively little effect on the total nitrogen content (*cf.*, 3, 5). The number of environmental factors common to the control of both total nitrogen and any of the more soluble nitrogen fractions would accordingly be lower. The results also support this hypothesis.

Further reference to Table I will show that the increases in the nitrogen of the different nitrogen fractions for equal increases in total nitrogen are not the same. These differences are best illustrated by means of the regression coefficients, given in the last line of the table, which represent the average

TABLE II

RELATION OF NITROGEN DISTRIBUTION TO TOTAL NITROGEN CONTENT

Station	Total nitrogen, % of dry matter	Percentage of total nitrogen		
		Total salt-soluble nitrogen	Alcohol-soluble protein nitrogen	Insoluble protein nitrogen
Nappan	1.540	38.8	31.2	30.0
Fredericton	1.738	38.1	33.5	28.4
Ste. Anne de la Pocatière	1.932	33.4	36.2	30.4
Ste. Anne de Bellevue	2.278	30.1	39.2	30.7
Lethbridge	2.294	35.4	38.9	25.7
Winnipeg	2.333	32.4	39.5	28.1
Brandon	2.357	31.4	40.8	27.7
Guelph	2.381	33.5	39.1	27.4
Ottawa	2.526	30.6	40.8	28.7
Lacombe	2.668	29.6	44.3	26.0
Beaverlodge	2.674	27.7	45.2	27.0
Gilbert Plains	2.687	30.0	39.6	30.3

unit increase of the nitrogen of the fractions per unit increase in total nitrogen. The alcohol-soluble fraction increases more than twice as fast as the insoluble fraction, and the latter increases about one and a half times as fast as the total salt-soluble fraction.

The effect of these differences in the regression coefficients on the distribution of the nitrogen at different total nitrogen levels is illustrated more clearly in Table II, in which the nitrogen of each of the three main fractions is reported as a percentage of total nitrogen. It is apparent that as the total nitrogen increases, the percentage of it in salt-soluble form decreases, the percentage in alcohol-soluble form increases, and the percentage in insoluble form remains relatively constant. These conclusions are identical with those of Bishop (1, 3), Hofman-Bang (7), and Fink and Kunisch (5).

Varietal Differences

Table III contains the mean values for each variety, together with the necessary differences between means required for a 5% level of significance.

TABLE III

MEAN TOTAL NITROGEN AND NITROGEN FRACTIONS FOR EACH VARIETY, AS PERCENTAGES OF DRY MATTER

Class	Variety	Total nitrogen	Salt-soluble			Alcohol-soluble protein nitrogen	Insoluble protein nitrogen
			Non-protein nitrogen	Protein nitrogen	Total		
Six-rowed, rough-awned	O.A.C. 21	2.242	.357	.418	.774	.883	.585
	Mensury, Ott. 60	2.303	.368	.414	.782	.899	.622
	Olli	2.215	.376	.418	.794	.772	.649
	Peatland	2.471	.366	.402	.768	1.028	.675
	Pontiac	2.278	.362	.373	.735	.918	.625
Six-rowed, smooth-awned	Nobarb	2.194	.327	.350	.677	.822	.695
	Regal	2.352	.348	.362	.710	.923	.718
	Velvet	2.372	.351	.367	.718	.945	.710
	Wisconsin 38	2.240	.329	.334	.663	.891	.686
Two-rowed, rough-awned	Charlottetown 80	2.298	.347	.404	.751	.962	.584
	Hannchen	2.215	.337	.378	.715	.882	.618
	Victory	2.228	.341	.382	.723	.922	.583
Mean, over all varieties		2.284	.350	.383	.734	.904	.646
Necessary difference, 5% level		.079	.010	.014	.028	.055	.036

It can be assumed that if the difference between the means for two varieties equals the necessary difference, the odds are 19 to 1 that a real difference between the varieties is operating to spread the means. It also follows that as the difference between means increases over that required for a 5% level of significance, the odds that a varietal difference exists also increase.

Inspection of the data will show that varietal differences exist with respect to total nitrogen and each of the fractions measured. This is the conclusion reached by Bishop (1, 3), who studied mainly the English two-rowed varieties

and found that the differences between them are confined largely to differences in salt-soluble and glutelin (insoluble) nitrogen. He noted, however, that the six-rowed variety F.112 differed markedly from the two-rowed varieties in its content of hordein (alcohol-soluble) nitrogen. Hofman-Bang (7) studied Danish two-rowed varieties and found that differences were most marked with respect to glutelin nitrogen. Fink and Kunisch (5) also confined their investigations to two-rowed varieties. They conclude that variety influences the nitrogen distribution, but state that varietal differences in glutelin content are for the most part so small that this can hardly serve as a varietal characteristic. If the latter conclusion is intended as a broad generalization, it is difficult to understand in view of the findings of earlier investigators. The truth appears to be that any given pair of varieties may differ with respect to one or more nitrogen fractions, and that a complete examination is required before it is possible to decide whether or not the varieties exhibit different nitrogen distribution patterns.

The varietal differences with respect to the various nitrogen fractions, shown by the data in Table III, are difficult to interpret owing to the complicating effect of varietal differences in total nitrogen content. For instance, it is impossible to tell by inspection whether Peatland is really characterized by high alcohol-soluble nitrogen, or whether the value for this fraction merely reflects the fact that Peatland is very high in total nitrogen content.

This difficulty can be overcome for the insoluble fraction by translating the values into percentages of total nitrogen, since it has been shown that the insoluble nitrogen represents a fairly constant percentage of the total nitrogen at all nitrogen levels. On the other hand, since salt-soluble nitrogen as percentage of total nitrogen decreases, and alcohol-soluble nitrogen as percentage of total nitrogen increases, with increasing nitrogen content, this stratagem will not serve for the treatment of these two fractions.

The problem is best solved by obtaining values representing the nitrogen distribution for each variety at equal total nitrogen contents. This process involves calculation of the coefficients of regression of the nitrogen of each fraction on total nitrogen. These coefficients are then used to adjust the varietal means for each nitrogen fraction to the values corresponding to a total nitrogen content of 2.28%, which figure represents the mean total nitrogen content of all varieties. The statistical analyses involved in carrying out this procedure, and in testing the validity of its application in the present instance, are described in the next section.

The adjusted varietal means for total salt-soluble, alcohol-soluble, and insoluble nitrogen are given in Table IV. It should be noted first that the solution of the problem is not perfect. If it were, the sum of the values for the three fractions, for each variety, would equal 2.28. The actual totals obtained are listed in the last column of the table. Since the maximum difference between the totals (0.07) represents only 3% of the mean value (2.28), it is apparent that the errors introduced by the method of calculation are of no great consequence.

The adjusted means given in Table IV present an entirely different picture from that presented by the original data (Table III). The order of the varieties with respect to each fraction is considerably changed, but differences between various pairs of varieties are still marked. A comparison of these differences, with those required for 5% level of significance, given in the last line of the table, leaves no room for doubt that the nitrogen distribution pattern at equal total nitrogen contents is a varietal characteristic.

TABLE IV

VARIETAL MEANS FOR NITROGEN FRACTIONS ADJUSTED BY CALCULATION TO VALUES CORRESPONDING TO TOTAL NITROGEN CONTENT OF 2.28%

Class	Variety	Total salt-soluble nitrogen	Alcohol-soluble protein nitrogen	Insoluble protein nitrogen	Check total, required 2.28
Six-rowed, rough-awned	O.A.C. 21	.780	.919	.597	2.296
	Mensury, Ott. 60	.779	.833	.617	2.229
	Olli	.803	.831	.668	2.302
	Peatland	.743	.869	.623	2.235
	Pontiac	.736	.923	.627	2.286
Six-rowed, smooth-awned	Nobarb	.689	.899	.720	2.308
	Regal	.710	.865	.699	2.274
	Velvet	.706	.870	.686	2.264
	Wisconsin 38	.663	.928	.698	2.289
Two-rowed, rough-awned	Charlottetown 80	.749	.950	.580	2.279
	Hannchen	.724	.940	.637	2.301
	Victory	.734	.970	.598	2.302
Mean, over all varieties		.735	.900	.646	2.28
Necessary difference, 5% level		.026	.015	.029	

The most interesting feature of the results now appears to be that there are indications of differences between the three classes of varieties. The three two-rowed varieties are higher in alcohol-soluble protein nitrogen and lower in insoluble protein nitrogen than any of the six-rowed varieties; and the four smooth-awned six-rowed varieties are lower in salt-soluble nitrogen and higher in insoluble protein nitrogen than any of the five rough-awned six-rowed varieties. These differences are particularly interesting in view of the fact that, in comparison with the rough-awned six-rowed varieties, the two-rowed varieties yield higher malt extracts, and the four smooth-awned varieties yield lower malt extracts, and all but Velvet are also lower in enzymatic activity.

It should be borne in mind, however, that owing to the small number of varieties studied and the variation within classes, the average differences between classes cannot be considered statistically significant. Nevertheless, the data do indicate that differences exist between classes, and further investigation with a larger number of varieties might serve to substantiate this hypothesis. This is particularly true with respect to differences between six-

and two-rowed varieties, since Bishop (3, 4) has already pointed out that his data show that the six-rowed varieties are lower in hordein nitrogen than the two-rowed varieties.

On the other hand, it seems much less probable that further investigation will show that differences exist between the rough- and smooth-awned six-rowed varieties. The members of the latter class at present available for study in Canada were developed from simple or multiple crosses, in which the smooth-awned variety Lion was one of the original parents and the other parents were rough-awned. As a group they have proved less satisfactory for malting, and this is attributed to carrying over in the progeny of undesirable characteristics of the parent Lion. It may be that the nitrogen distribution pattern of the four smooth-awned varieties studied in this investigation is also attributable to the Lion parent. There is no good reason for believing, however, that this apparent association between the smooth-awned character and certain biochemical properties cannot be broken, and it therefore seems unwise to generalize about differences between groups of varieties which are classified solely by means of the presence or absence of barbs on the awns.

Statistical Analyses*

The variances of the data for each determination were analyzed into portions resulting from (i) average differences between varieties; (ii) average differences between stations; and (iii) remainder. The last portion results not only from variations caused by a true interaction between stations and varieties, but also from variations caused by soil heterogeneity within stations, and by sampling and analytical errors. It therefore provides an adequate criterion for testing the significance of differences between station and varietal means.

TABLE V

ANALYSIS OF VARIANCE FOR TOTAL NITROGEN AND NITROGEN FRACTIONS: MEAN SQUARES

Variance due to	Degrees of freedom	Total nitrogen	Salt-soluble			Alcohol-soluble protein nitrogen	Insoluble protein nitrogen
			Non-protein nitrogen	Protein nitrogen	Total		
Varieties	11	.07883**	.00290**	.00929**	.02022**	.05094**	.02994**
Stations	11	1.65078**	.00877**	.03145**	.05943**	.59097**	.11951**
Remainder	121	.00934	.00058	.00121	.00119	.00447	.00198

NOTE: *In this and later tables, ** denotes that the 1% level, and * that the 5% level of significance is attained.*

The mean squares obtained by the analyses of variance are reported in Table V. Examination of these shows that by far the largest portion of the variance of each set of data is attributable to differences in the average performance of all varieties at different stations, and that there is overwhelming

* *All methods referred to in this section are admirably described by Snedecor (10).*

evidence that significant differences exist between station means. Considerable portions of the variance also result from differences in the average performance, over all stations, of the individual varieties, and since these portions are significantly greater than the corresponding remainders, it is apparent that varietal differences exist with respect to total nitrogen and each of the nitrogen fractions studied.

The necessary differences between varietal means required for a 5% level of significance, given in the last line of Table III, were calculated by dividing the remainder mean square by 12, taking the square root, and multiplying $2\sqrt{2}$.

The data for total nitrogen and each of the nitrogen fractions were next subjected to analyses of variance and covariance. The resulting correlation and regression coefficients are given in Table VI. Amongst the correlation coefficients for varietal means only that for alcohol-soluble nitrogen and total nitrogen proved to be significant. It is thus apparent that there is some tendency for varieties that are higher in total nitrogen to be higher in alcohol-soluble nitrogen also. The relation, however, is not particularly close, so that exceptions to this generalization are to be expected. There is no indication that varieties that are higher in total nitrogen will also tend to be higher in other nitrogen fractions.

TABLE VI

ANALYSES OF VARIANCE AND COVARIANCE FOR TOTAL NITROGEN AND EACH NITROGEN FRACTION

Coefficient for	Degrees of freedom	Salt-soluble			Alcohol-soluble protein nitrogen	Insoluble protein nitrogen
		Non-protein nitrogen	Protein nitrogen	Total		
Correlation coefficients						
Varieties	11	.398	.129	.238	.811**	.369
Stations	11	.823**	.736**	.851**	.985**	.926**
Remainder	121	.162	.262**	.382**	.851**	.600**
Total	143	.600**	.575**	.703**	.964**	.817**
Regression coefficients						
Varieties	11	.076	.004	.120	.652	.227
Stations	11	.060	.102	.161	.589	.249
Remainder	121	.040	.094	.135	.589	.276
Total	143	.060	.099	.158	.592	.250

All of the correlation coefficients for station means are significant and thus show, as would be expected on common-sense grounds, that as the mean total nitrogen content of the varieties increases, the amount of each nitrogen fraction also tends to increase. The high correlation coefficient for alcohol-soluble nitrogen indicates that many of the environmental factors which control total nitrogen content also control the amount of alcohol-soluble nitrogen. The number of factors common to the control of total nitrogen and any of the other fractions is apparently lower. Differences in the degree

of association between total nitrogen and each fraction are further illustrated by differences in the correlation coefficients calculated for remainders. That for the alcohol-soluble fraction is by far the highest, whereas that for non-protein nitrogen does not even attain the 5% level of significance.

Differences between the regression coefficients for the various nitrogen fractions have been discussed in the previous section. It is necessary to note here only that the agreement between the coefficients for stations and remainder is excellent for the alcohol-soluble fraction and moderately satisfactory for the insoluble and total salt-soluble fractions.

It appeared that a more useful comparison of varietal differences in nitrogen distribution might be obtained by making these at equal total nitrogen contents. The analyses of variance and covariance showed that the necessary calculations, (*i.e.*, correction of varietal means by means of the regression coefficients calculated from the remainders) might logically be undertaken for the alcohol-soluble, insoluble, and total salt-soluble fractions.

Before proceeding further, it was necessary to demonstrate that the regression coefficients for individual varieties did not differ significantly, since if they did, it is obvious that calculation of varietal means corresponding to equal total nitrogen contents would introduce a complicating factor whose effect could not easily be interpreted. The homogeneity of the varietal regression coefficients was accordingly tested by means of an analysis of residual inter-station, intra-varietal variance, the results of which are given in Table VII. Since the mean squares resulting from differences in varietal regression coefficients are not even as large as those from deviations from individual regressions, it is apparent that varietal regression coefficients do not differ significantly. The validity of using one regression coefficient for adjusting the means for all varieties was thus demonstrated. The best estimate of the average regression for each fraction on total nitrogen appeared to be that obtained from the remainders, and this was accordingly used in computing the adjusted means reported in Table IV.

In order to complete the examination of the data it was necessary to investigate the significance of differences among the adjusted varietal means. Mean squares, adjusted for differences in total nitrogen, were accordingly calculated and their significance was determined in the usual way. The

TABLE VII

TEST OF HOMOGENEITY OF VARIETAL REGRESSION COEFFICIENTS BY ANALYSIS OF RESIDUAL VARIANCE

Variance due to	Degrees of freedom	Mean squares		
		Total salt-soluble nitrogen	Alcohol-soluble protein nitrogen	Insoluble protein nitrogen
Differences among varietal regression coefficients	11	.00185	.00178	.00224
Deviations from individual varietal regressions	120	.00237	.00269	.00264

resulting statistics are reported in Table VIII. Since the residual mean squares resulting from average differences between varieties are significantly greater than those from the corresponding remainders, the statistics demonstrate that at equal total nitrogen contents the varieties differ in their nitrogen distribution patterns.

TABLE VIII

TEST OF SIGNIFICANCE OF VARIANCE IN NITROGEN FRACTIONS ADJUSTED FOR DIFFERENCES IN TOTAL NITROGEN CONTENT

Variance due to	Degrees of freedom	Residual mean squares		
		Total salt-soluble nitrogen	Alcohol-soluble protein nitrogen	Insoluble protein nitrogen
Varieties	11	.01908**	.01761**	.02596**
Stations	11	.01645**	.01752**	.01712**
Remainder	120	.00103	.00124	.00128

In carrying out the calculations involved in adjusting the varietal means, it has been assumed that the regressions are linear rather than curvilinear. In order to test this hypothesis, scatter diagrams were prepared. These showed little indication of curved regressions, and it was decided by inspection that owing to the scatter of the points it was improbable that a significant improvement in fit would be obtained by using curvilinear regression lines. Moreover, it seemed apparent that even if a significant improvement in fit were obtained, the interpretation of the more important features of the experimental data would not be facilitated.

Acknowledgments

The success of the present investigation, and of similar ones to be described in following papers, depends primarily upon the suitability of the samples available for study. For this reason, the authors are greatly indebted to members of the staffs of the various Dominion Experimental Farms and Agricultural Colleges, previously listed, and particularly to Mr. P. R. Cowan of the Central Experimental Farm, Ottawa, for co-operation in making available for study an excellent set of barley samples.

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THE APPLICATION OF SEROLOGICAL METHODS TO THE DIFFERENTIATION OF CLOSELY RELATED SMUT FUNGI[1]

By E. C. Beck[2]

Abstract

Serological methods were applied in an attempt to differentiate a number of closely related members of the family Ustilaginaceae. The results of two series of reciprocal precipitin-ring tests showed that different genera and species of the same family were satisfactorily differentiated by this technique; so also were compatible cultures of the same species, where no detectable differences existed, other than the necessity of the haploid counterparts being brought together on the appropriate host plant to induce the diploid phase, and subsequent infection of the host. A parent culture and its mutant that were different morphologically but alike in their pathogenicity, were the only ones that could not be differentiated by this technique. Reciprocal absorption tests were applied to these two fungi, but the powder of either culture absorbed the antibodies of both from the immune sera. Optimal proportions of antigen and antibody were determined, but could not be applied in absorption tests because of the dilution of antisera. Agglutination tests were attempted but were unfruitful.

Introduction

Immunological studies have contributed a fund of information to perplexing questions of taxonomic relationships in groups of fungi and higher plants, where forms so closely resemble one another as to cause doubt regarding their relation. In view of the fact that some of the more recent serological investigations have thrown new light on the difficulties encountered in former studies, it was decided to reinvestigate the usefulness of the precipitin-ring test as an additional taxonomic criterion in differentiating a group of closely related smut fungi, employing, wherever possible, improvements in technique as reported in the literature. The smuts were chosen as test organisms because while they are highly obligate in their parasitic relations, many of them can be cultured on artificial media in their haploid stage. Within the group, therefore, it is possible to choose as experimental material members related in widely different degree, including different genera, species, physiological forms and even sexually compatible haplonts of heterothallic species. The precipitin-ring test was undertaken because of the encouraging results secured in earlier work (5), and because of the possibility that it might furnish some information with regard to the standardization of components to be used in other serological tests, applied subsequently.

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Co-operative contribution from the Department of Botany and the School of Hygiene, University of Toronto, Ontario. Part of a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the University of Toronto.
[2] *Professor of Bacteriology, Ontario Agricultural College, Guelph, Ont. Formerly lecturer, Department of Botany, University of Toronto.*

The Precipitin-ring Test

Materials and Methods

The cultures employed were all members of the family Ustilaginaceae, representing different genera, species, compatible cultures* from the same chlamydospore, and one mutant and its parent culture. *Ustilago avenae* (Pers.) Jensen, *Sorosporium reilianum* (Kuhn) McAlpine, and *Sphacelotheca sorghi* (Lk.) Clinton, were procured from the Baarn Type Culture Collection, Baarn, Holland. *Ustilago zeae* (Beckm.) Ung. and *U. hordei* (Pers.) K. & S. were supplied through the courtesy of Dr. J. J. Christensen, Minnesota University. The three cultures of *U. zeae* were isolated from a single chlamydospore, as were the two cultures of *U. hordei*. *U. hordei* C. was a mutant from *U. hordei* B.

The medium recommended by Stakmann *et al.* (35)† as being especially suitable for the growth of this group of fungi was used throughout the experiments. Tests conducted to determine the most suitable pH of the medium indicated that the cultures were extremely tolerant with respect to pH and gave abundant growth on media adjusted to a range of pH 4.2 to 10. The optimum was approximately pH 6.8, and the medium did not need re-adjustment. All cultures were grown in eight-ounce medicine bottles containing about 30 cc. of the medium, which had been allowed to solidify over the maximum surface, and were incubated at room temperature for three to four weeks. Petri-dish cultures were found to be unsatisfactory because of the rapid drying of the medium, the frequency of contamination during such a long incubation period, and the relatively small yield of material. Fungal mats were peeled from the surface of the agar by means of a stout hooked needle, in such a way as to avoid bringing with them particles of the medium. Where this was unavoidable, the mats were scraped with a clean scalpel. The fungal mats were piled loosely in open Petri dishes and were dried in desiccators over sulphuric acid. Later, it was found more satisfactory to dry them at 45° C. in an incubator containing a large dish of anhydrous calcium chloride. The dry, crisp material was then ground in chemically clean mortars by means of a hand pestle, until fine enough to pass through a sieve of 100 mm. mesh. The powders were stored in sealed containers in the refrigerator. Powders prepared in this way have been kept for years without deterioration. The average net yield of dried material from a culture varied from 0.069 gm. to 0.935 gm.

Preparation of the Extracts

Injection fluids used for immunization were prepared by extracting 0.3 gm. of the dry powder in 10 cc. of 0.85% sodium chloride solution for 18 to 24 hr. in the refrigerator, after which the whole was centrifuged; the clear supernatant

* *Cultures which when brought together on a suitable host will give rise to the diploid phase and produce infection.*

† *Potato extract from 400 gm. of potatoes in 1 litre of water; 1.8% agar; 1% dextrose; 1% sucrose; and 1% malt extract.*

fluid was used for intravenous injection, the sediment was resuspended in 10 cc. of saline and used concomitantly for intraperitoneal injections.

Antigens were prepared by extracting 0.3 gm. of the dry powder in 15 cc. of saline for 18 to 24 hr. in the refrigerator, followed by centrifuging until the supernatant fluid was crystal clear. Such fluids, which constituted the stock antigens, had a dilution of 1 : 50, and from them were prepared serial dilutions.

Injection fluids and antigens prepared in this manner were tested for the presence of proteins by the biuret test, and all gave a positive reaction.

Immunization of Rabbits

A small amount of blood was drawn from a marginal ear vein of each rabbit prior to immunization, and tested against the extract that was to be used later for injection. Since these normal sera were negative, the protocols of the tests have been omitted.

Immunization was accomplished by administering 2 cc. of the supernatant extract intravenously plus 3 cc. of the resuspended powder intraperitoneally, daily for three days. This was followed by a rest period of four days, after which the same treatment was repeated for three days. The animals were then rested for nine days, starved, and bled from the ear vein on the tenth day. From 30 to 50 cc. of blood was drawn, and the samples were stored in the refrigerator overnight. The next morning they were centrifuged, and the serum was removed to sterile vaccine bottles.

Setting up and Reading the Tests

Small precipitin tubes 10 mm. by 750 mm. were used, in order to economize with the antiserum. All glassware was cleaned in strong cleaning solution* before use. Saline used for dilution purposes and for controls was freshly prepared, as were the antigens. Undiluted antiserum (0.1 cc.) was used throughout the tests. Antigen dilutions were prepared in separate tubes, and by means of separate capillary pipettes, 0.1 cc. of the appropriate antigen dilution was carefully run over the same amount of serum.

Saline plus antigen and serum plus saline controls were included in all tests, but since they were negative throughout, they have been omitted from the tables.

All racks of tubes were incubated for one hour in a constant temperature water-bath at 45° C., after which they were read in a specially lighted chamber. Shorter periods of incubation were found to be unsatisfactory, since some of the reactions appeared more slowly than others. Reading the tests a second time after storing them in the refrigerator overnight was also unsatisfactory, because the diffusion of the components interfered with the reading.

In recording the results, "4" was used to indicate the maximum reaction, and in such instances there was not a clearly defined precipitin "ring" at the junction of the serum and antigen, but an opaque, fine and uniform preci-

* *Potassium dichromate saturated solution, 500 cc.; crude concentrated sulphuric acid, 800 cc.*

pitate throughout the mass; "3" indicates the presence of a continuous ring at the interphase, while "2" and "1" represent a definite ring less perfectly expressed. The sign ± is employed to indicate a doubtful reaction which could not be considered as a typical positive, although in a few instances such might have become positive with prolonged incubation.

The titre of an antiserum is taken as the highest dilution in which a continuous precipitin ring occurs between undiluted antiserum and its homologous antigen.

Results of the Precipitin Tests

The results of these tests are presented in Table I, from which it is seen that potent antisera were produced for all extracts, the lowest titre being 1 : 3200, while six antisera possessed a titre of 1 : 12,800. Not only were the antisera high in titre, but they showed a high degree of specificity. The three extracts of monosporidial cultures of *Ustilago zeae* were distinguished as separate serological entities; so also were *U. hordei* A and B, which were also monosporidial cultures from a single chlamydospore. Extracts of the mutant culture *U. hordei* C were indistinguishable from the parent culture *U. hordei* B, when tested reciprocally by this technique. The three antisera of *U. zeae* gave strong group reactions with *U. avenae* antigen, but the antiserum of *U. avenae* was not reciprocally reactive with the antigens of *U. zeae*. The antiserum of *Sorosporium reilianum* gave marked group reactions with most of the antigens of other cultures, but the antisera of these cultures were not proportionately reactive with the antigen of *Sorosporium*.

Interpreting these results on the basis of the conventional reading of the precipitin and agglutination reactions in general practice, it is concluded that the differences between titres and non-specific reactions are great enough to serve as a basis for identification of the members of this group of fungi. *Ustilago hordei* B and its mutant were the only exceptions.

Supplementary Tests

Second Series of Precipitin Tests

In this series of tests, modifications were made in the preparation of the injection fluids and antigens, and in the immunization of the rabbits. In all other respects the procedure was similar to that already described.

The injection fluids were prepared by regrinding 0.3 gm. of the powder for about 10 min. with small amounts of saline, until 6 cc. had been incorporated. The mortar was rinsed with 4 cc. of saline. The mixture was allowed to extract in the refrigerator for 18 to 24 hr., then centrifuged. The supernatant fluids remained turbid.

Antigens used in this series were prepared by regrinding 0.3 gm. of powder in 15 cc. of saline, followed by extraction and centrifuging as above. These extracts could not be used as antigens until they had been filtered through Seitz bacteria-proof filters to render them crystal clear.

The turbid fluids gave much more marked biuret reactions than the clear extracts. Extraction of the powders was also attempted with glycerine,

TABLE I

RESULTS OF FIRST SERIES OF PRECIPITIN TESTS

Antigens	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800
	U. zeae A antiserum									*U. zeae* B antiserum									*U. zeae* C antiserum								
U. zeae A	2	1	1	1	1	1	1	—	—	3	2	1	1	1	—	—	—	—	4	3	2	1	2	1	—	—	—
U. zeae B	1	1	—	—	—	—	—	—	—	4	1	1	1	1	3	3	3	1	4	3	2	2	1	±	—	—	—
U. zeae C	1	1	—	—	—	—	—	—	—	4	3	3	1	—	—	—	—	—	4	3	3	3	1	1	1	2	1
U. hordei A	1	1	—	—	—	—	—	—	—	2	1	±	—	—	—	—	—	—	4	3	3	1	±	—	—	—	—
U. hordei B	2	1	±	—	—	—	—	—	—	3	1	±	—	—	—	—	—	—	4	1	1	1	—	—	—	—	—
U. hordei C	3	1	1	—	—	—	—	—	—	4	1	±	1	—	—	—	—	—	4	1	1	±	—	—	—	—	—
U. avenae	4	2	1	1	—	—	—	—	—	4	3	2	1	1	—	—	—	—	4	3	3	2	1	1	—	—	—
S. reilianum	2	1	1	—	—	—	—	—	—	4	1	1	—	—	—	—	—	—	3	±	±	1	1	—	—	—	—
S. sorghi	1	1	1	±	±	—	—	—	—	1	—	—	—	—	—	—	—	—	4	1	1	1	±	—	—	—	—
	U. hordei A antiserum									*U. hordei* B antiserum									*U. hordei* C antiserum								
U. zeae A	4	1	1	1	—	—	—	—	—	4	1	—	—	—	—	—	—	—	2	1	±	±	±	±	±	—	—
U. zeae B	4	2	1	—	—	—	—	—	—	4	1	—	—	—	—	—	—	—	3	2	1	—	±	±	—	—	—
U. zeae C	4	2	1	—	—	—	—	—	—	4	±	±	—	—	—	—	—	—	3	2	1	±	±	±	±	—	—
U. hordei A	4	3	3	1	1	1	1	±	—	4	3	2	2	1	—	—	—	—	2	1	1	1	1	1	—	—	—

Concluded on page 396

TABLE I—*Concluded*

RESULTS OF FIRST SERIES OF PRECIPITIN TESTS—*Concluded*

Antigens	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800
	U. hordei A antiserum—*Con.*									*U. hordei* B antiserum—*Con.*									*U. hordei* C antiserum—*Con.*								
U. hordei B	4	2	2	1	1	—	—	—	—	4	2	2	2	2	1	1	1	1	4	2	2	1	1	1	1	±	—
U. hordei C	4	3	3	1	1	±	—	—	—	4	2	2	1	±	1	1	1	±	4	2	2	2	1	1	1	1	1
U. avenae	4	3	1	1	—	—	—	—	—	4	2	1	1	±	—	—	—	—	4	3	1	—	—	—	—	—	—
S. reilianum	4	1	1	—	—	—	—	—	—	4	1	—	—	—	—	—	—	—	2	1	1	—	—	—	—	—	—
S. sorghi	4	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	±	±	—	—	—	—	—
	U. avenae antiserum									*Sorosporium reilianum* antiserum									*Sphacelotheca sorghi* antiserum								
U. zeae A	3	2	1	—	—	—	—	—	—	4	2	1	1	—	—	—	—	—	4	1	1	—	—	—	—	—	—
U. zeae B	2	1	1	—	—	—	—	—	—	4	3	2	1	—	—	—	—	—	3	—	—	—	—	—	—	—	—
U. zeae C	2	1	1	—	—	—	—	—	—	3	2	2	1	1	—	—	—	—	3	—	1	—	—	—	—	—	—
U. hordei A	1	1	1	—	—	—	—	—	—	3	2	2	2	2	1	—	—	—	4	—	—	—	—	—	—	—	—
U. hordei B	3	2	1	—	—	—	—	—	—	4	3	2	2	2	1	—	—	—	4	—	1	±	—	—	—	—	—
U. hordei C	3	2	1	—	—	—	—	—	—	4	2	2	1	1	—	—	—	—	4	1	1	±	—	—	—	—	—
U. avenae	4	4	3	2	2	2	1	1	1	4	3	3	2	1	1	—	—	—	4	3	1	±	—	—	—	—	—
S. reilianum	3	1	1	—	—	—	—	—	—	4	3	2	2	1	1	1	1	±	4	1	—	—	—	—	—	—	—
S. sorghi	2	1	1	—	—	—	—	—	—	4	1	1	1	1	—	—	—	—	4	2	2	2	2	2	1	1	1

All antisera were used undiluted.

4 = very heavy precipitate throughout serum and antigen; 3 = a continuous ring at the interphase; 2 and 1 = a definite ring less perfectly expressed; ± = doubtful reaction.

hypertonic saline (2.55% sodium chloride), and 1% sodium carbonate, but these methods were abandoned since they offered some disadvantages without compensating advantages.

Immunization

The turbid extract (1 cc.) was used as the initial intravenous injection and no intraperitoneal injection was made. Of seven rabbits treated in this way, five succumbed within 48 hr. Another group of animals was started with an initial intravenous dose of 0.25 cc. of the turbid fluid, followed by five subsequent doses of 0.5 and 1.0 cc., and three injections of 1.5 cc. each, without intraperitoneal injections. After resting the animals for four days, four intraperitoneal injections of the resuspended sediment were given in 2-cc. amounts. This was followed by the procedure already described.

Results

The results of this series of precipitin tests are given in Table II, from which it will be seen that, while the results are parallel to and in general agreement with the first series, the titres throughout are much lower, as are the non-specific or group reactions. Eight of the nine antisera possessed a titre of 1 : 1600 and one titre of 1 : 3200, while the highest group reaction occurred in an antigen dilution of 1 : 400, and the degree of specificity is approximately the same in the two series. It would seem that the difference in the immunizing procedure might account, in part at least, for the fall in titre in the second series of tests, since the animals lost considerahle weight during immunization.

Additional Refinements in Technique

In order to differentiate still more sharply the components of the series studied, the following three refinements in technique were attempted; first, to increase the antibody content of immune sera; second, to determine the optimal proportions of antigen and antibody; and third, reciprocal absorption tests.

Attempts to Increase the Antibody Content of Immune Sera

Various workers have noted a marked rise in the titre of an antiserum resulting from a single injection of the immunizing fluid administered several days after the last injection of the immunizing series. It was felt that if this condition could be achieved, sharper differentiation of the various fungi might result. In order to test the possibility, seven of the rabbits used in the second series of tests were each given one intraperitoneal injection, consisting of 2.5 cc. of the resuspended appropriate fungus powder. The injection was given 12 days after the last injection of the regular series, or two days after bleeding for the regular tests. After a rest period of 12 days, sufficient blood was drawn from the ear vein to test against the homologous antigen. The results given in Table III indicate that no secondary stimulation resulted. They do, however, confirm repeated observations with regard to the rapid fall in titre of the antisera, both *in vivo* and *in vitro*. An examination of the data contained in Table III reveals a reduction in titre from 1 : 1600 to 1 : 200 after 24 days "storage *in vivo*".

TABLE II

RESULTS OF SECOND SERIES OF PRECIPITIN TESTS

Antigens	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800
	U. zeae A antiserum									*U. zeae* B antiserum									*U. zeae* C antiserum								
U. zeae A	4	4	3	2	1	1	±	±	±	3	1	—	—	—	—	—	—	—	3	2	1	—	—	—	—	—	—
U. zeae B	2	1	—	—	—	—	—	—	—	4	3	2	1	1	1	±	±	—	4	3	2	1	±	—	—	—	—
U. zeae C	4	3	2	1	—	—	—	—	—	2	2	1	—	—	—	—	—	—	4	4	3	2	1	1	—	—	—
U. hordei A	4	3	2	1	—	—	—	—	—	3	1	—	—	—	—	—	—	—	3	2	1	—	—	—	—	—	—
U. hordei B	4	3	—	—	—	—	—	—	—	3	2	—	—	—	—	—	—	—	3	2	—	—	—	—	—	—	—
U. hordei C	3	1	—	—	—	—	—	—	—	3	1	—	—	—	—	—	—	—	3	2	—	—	—	—	—	—	—
U. avenae	3	2	1	—	—	—	—	—	—	2	1	—	—	—	—	—	—	—	4	3	—	—	—	—	—	—	—
S. reilianum	2	3	1	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	4	3	—	—	—	—	—	—	—
S. sorghi	4	3	2	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	4	3	1	—	—	—	—	—	—
	U. hordei A antiserum									*U. hordei* B antiserum									*U. hordei* C antiserum								
U. zeae A	3	2	1	—	—	—	—	—	—	3	2	1	±	—	—	—	—	—	2	1	—	—	—	—	—	—	—
U. zeae B	4	3	2	1	—	—	—	—	—	4	3	2	1	—	—	—	—	—	3	1	—	—	—	—	—	—	—
U. zeae C	3	2	1	—	—	—	—	—	—	3	2	1	—	—	—	—	—	—	1	1	±	—	—	—	—	—	—
U. hordei A	4	3	2	1	1	1	±	—	—	2	1	±	—	±	—	—	—	—	3	2	1	1	1	—	—	—	—

Concluded on page 399

TABLE II—*Concluded*

RESULTS OF SECOND SERIES OF PRECIPITIN TESTS—*Concluded*

Antigens	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12,800
	U. hordei A antiserum—*Con.*									*U. hordei* B antiserum—*Con.*									*U. hordei* C antiserum—*Con.*								
U. hordei B	3	2	—	±	±	—	—	—	—	3	2	1	1	1	1	±	—	—	4	3	2	1	1	±	±	—	—
U. hordei C	3	2	—	—	—	—	—	—	—	4	3	1	1	1	1	±	±	—	4	3	2	1	1	1	1	—	—
U. avenae	3	2	1	1	—	—	—	—	—	4	3	2	1	—	—	—	—	—	4	3	2	—	—	—	—	—	—
S. reilianum	2	1	—	—	—	—	—	—	—	3	2	1	±	—	—	—	—	—	3	2	1	±	—	—	—	—	—
S. sorghi	3	—	—	—	—	—	—	—	—	3	2	1	—	—	—	—	—	—	3	2	—	—	—	—	—	—	—
	U. avenae antiserum									*Sorosporium reilianum* antiserum									*Sphacelotheca sorghi* antiserum								
U. zeae A	2	1	±	—	—	—	—	—	—	3	1	—	—	—	—	—	—	—	2	1	—	—	—	—	—	—	—
U. zeae B	2	1	±	—	—	—	—	—	—	3	1	—	—	—	—	—	—	—	3	2	1	—	—	—	—	—	—
U. zeae C	3	2	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	3	2	1	—	—	—	—	—	—
U. hordei A	3	2	1	—	—	—	—	—	—	2	1	—	—	—	—	—	—	—	2	1	—	—	—	—	—	—	—
U. hordei B	3	2	1	±	—	—	—	—	—	3	2	1	—	—	—	—	—	—	2	1	—	—	—	—	—	—	—
U. hordei C	3	2	1	±	—	—	—	—	—	2	±	±	—	—	—	—	—	—	3	2	1	—	—	—	—	—	—
U. avenae	4	3	2	1	1	1	±	—	—	4	3	2	1	±	—	—	—	—	2	1	±	—	—	—	—	—	—
S. reilianum	3	2	1	—	—	—	—	—	—	3	2	1	1	1	1	—	—	—	2	1	—	—	—	—	—	—	—
S. sorghi	2	1	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	4	3	2	1	1	1	±	±	—

All antisera were used undiluted.

4 = very heavy precipitate throughout serum and antigen; 3 = a continuous ring at the interphase; 2 and 1 = a definite ring less perfectly expressed; ± = doubtful reaction.

TABLE III

ATTEMPT TO INCREASE SERUM TITRE BY SECONDARY STIMULATION

Antisera + homologous antigens

Antisera	Antigen dilutions						Original titre
	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	
U. zeae A	3	2	1	—	—	—	1 : 1600
U. zeae B	3	2	±	—	—	—	1 : 1600
U. zeae C	3	2	1	—	—	—	1 : 1600
U. hordei A	3	2	2	±	—	—	1 : 1600
U. hordei B	3	2	1	—	—	—	1 : 1600
U. hordei C	2	1	1	—	—	—	1 : 3200
S. reilianum	3	2	1	—	—	—	1 : 1600

Optimal Proportions of Antigen and Antibody

Before conducting reciprocal absorption tests to determine the serological relation of *U. hordei* B and its mutant *U. hordei* C, it was deemed advisable to determine the optimal proportion of antigen to undiluted antiserum, in the hope that such tests would furnish some guide towards the technique of effective absorption. Dean and Webb (11) pointed out the usefulness of the method for the quantitative estimation of either antigen or antibody, and Ramon (33) demonstrated the value of the test in the titration of diphtheria antitoxin. Smith (34), employing this technique, concluded that *in vitro* tests for determining the potency of antipneumococcus serum were more accurate and more rapid than the laborious and expensive animal tests. In conducting absorption tests it is important to know the optimal proportions of the reagents, since most immunological reactions with a relative excess of one or other of the test fluids may show "specific inhibition" preventing the visible expression of the reaction. This phenomenon has been attributed by Opie (30, 31) to an excess of antigen, which dissolves the precipitate formed by the union of precipitin and its antigen.

The antisera were used undiluted against graded dilutions of antigen made up to the same volume of serum with 0.85% saline, as indicated in Table IV. Saline and antigen were added to the tubes first and mixed, followed by 0.5 cc. of the antiserum, which was carefully run down the side of the tube. The tubes were left undisturbed at room temperature until the "critical tube" or "indicator tube" was noted. The tests were then incubated at 45° C. for one hour before making the final readings. On addition of *U. hordei* B serum to graded dilutions of the homologous antigen, precipitation occurred in one and one-half minutes in the tube containing 0.05 cc. of antigen, or in an antigen dilution of 1 : 10, while precipitates formed more slowly on either side of this dilution. When *U. hordei* C antiserum was added to 0.4 cc. of its antigen (1 : 1.25 dilution), precipitation occurred at once. The optimal proportions of antigen and antibody (as indicated by the "critical tube", using dilutions of 1:50 stock antigen) were 1 : 10 and 1 : 1.25 respectively,

and when these dilutions were departed from, in the direction of either antigen or antibody excess, precipitation took place more slowly. Dean and Webb pointed out that most rapid precipitation occurred in the mixture in which antigen and antibody were present in such amounts that none of either reagent remained uncombined, or that only a trace remained detectable in the supernatant fluid.

TABLE IV

OPTIMAL PROPORTIONS OF ANTIGEN AND ANTIBODY

Schedule									Controls	
Tubes	1	2	3	4	5	6	7	8	9	10
Saline	0	.1	.2	.3	.4	.45	.47	.49	.5	.5
Antigen	.5	.4	.3	.2	.1	.05	.03	.01	.5	.0
Serum	.5	.5	.5	.5	.5	.5	.5	.5	0	.5
Reactions										
U. hordei B antigen, 1 : 50 *U. hordei* B serum, undiluted	–	–	–	2	3	3*	3	2	–	–
U. hordei C antigen, 1 : 50 *U. hordei* C serum, undiluted	3	3†	3	2	2	2	–	–	–	–

* *"Indicator tube" appeared in 1¾ min.*
† *"Indicator tube" immediate reaction.*

Reciprocal Absorption

This test was employed as a final means of determining whether *U. hordei* B and its mutant *U. hordei* C were serologically identical, since they have proved to be more or less reciprocally reactive to the same degree, as exemplified by the precipitin-ring test.

The optimal proportions of antigen and antibody, as previously determined, were found to be unsatisfactory for application in the absorption tests, since the dilution of the serum by the antigen rendered it unsuitable for the ring test. It was also found necessary to employ the powder instead of the powder extract, in order to effect satisfactory absorption of the antibodies. To determine the actual relation of the mutant culture and its parent, absorption was conducted in the following manner: to 0.4 gm. of the fungus powder, 1.6 cc. of saline was added. These were ground together in a mortar, removed to the refrigerator and allowed to extract for 18 to 24 hr., after which the suspension was added to 2 cc. of the serum to be absorbed. These serum-extract mixtures were incubated in a water-bath at 56° C. for 2 hr. with frequent shaking. They were then stored in the refrigerator for 18 hr. before being centrifuged, in order to secure clear serum. Such treatment did not, however, produce the crystal-clear serum needed for the tests, and it was found necessary to filter the supernatant fluid through Seitz

bacteria-proof filters. During absorption, a fine permanent turbidity appeared which was identical with a "4" precipitin reaction.

TABLE V

RECIPROCAL ABSORPTION OF PRECIPITINS

From *U. hordei* B antiserum and *U. hordei* C antiserum

Antiserum	Suspension	Antigen	Antigen dilutions 1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600
U. hordei B absorbed by	*U. hordei* B *vs.*	*U. hordei* B	–	–	–	–	–	–
U. hordei B absorbed by	*U. hordei* B *vs.*	*U. hordei* C	–	–	–	–	–	–
U. hordei B absorbed by	*U. hordei* C *vs.*	*U. hordei* B	–	–	–	–	–	–
U. hordei B absorbed by	*U. hordei* C *vs.*	*U. hordei* C	–	–	–	–	–	–
U. hordei C absorbed by	*U. hordei* B *vs.*	*U. hordei* B	–	–	–	–	–	–
U. hordei C absorbed by	*U. hordei* B *vs.*	*U. hordei* C	–	–	–	–	–	–
U. hordei C absorbed by	*U. hordei* C *vs.*	*U. hordei* B	–	–	–	–	–	–
U. hordei C absorbed by	*U. hordei* C *vs.*	*U. hordei* C	–	–	–	–	–	–
Controls								
U. hordei B *vs.* *U. hordei* B		Antigens	4	3	3	2	1	±
U. hordei B *vs.* *U. hordei* C		Antigens	4	3	3	2	1	1
U. hordei C *vs.* *U. hordei* B		Antigens	4	3	2	1	±	–
U. hordei C *vs.* *U. hordei* C		Antigens	4	3	2	2	1	1
Diluted antiserum—1 : 1								
U. hordei C *vs.* *U. hordei* C		Antigens	2	1	1	±	–	–
U. hordei B *vs.* *U. hordei* B		Antigens	2	1	1	–	–	–

As a precaution against misinterpretation resulting from dilution of the antiserum by the absorbing material, serum diluted with an equal volume of saline was tested with standard serially diluted antigen. The reduction in reaction of serum by dilution is shown in Table V.* The schedule of reciprocal absorption tests is also given in Table V.

It was concluded from the results of these tests that these antisera were totally non-specific, being completely absorbed reciprocally by the antigens, while the controls remained positive. Or, in other words, these two sera contained major antibodies indicating that the two cultures were serologically identical. If, then, these two cultures are to be considered as separate entities, some criterion other than the precipitin-ring and absorption tests must be employed.

The Agglutination Test

In taxonomic studies dealing with phytopathogenic bacteria, the agglutination technique has been applied with results that would seem to justify its use as a "standard" criterion in identification and classification. However,

* *It should be remembered that absorption sometimes reduces the titre of a serum for its homologous antigen.*

a more careful perusal of the literature reveals many instances in which the test was not suitable.

The relative simplicity of the test is so advantageous that repeated attempts have been made by the author to apply the method in studies of fungus relations.

The preparation of a suitable antigen has been the chief obstacle in the application of this test, but the antigen as prepared in the second series of precipitin tests, before filtration to clear it, seemed to present a suspension analogous to a bacterial suspension, insofar at least as it remained uniformly turbid.

To serially diluted antiserum an equal volume of undiluted antigen (stock solution 1:50) was added. The tubes were shaken and incubated at 37° C. for one hour, after which they were read in a specially lighted rack as used for reading the precipitin tests. There was no indication of a typical agglutination reaction, all tubes showed varying degrees of uniform turbidity which remained in a state of permanent suspension. The Widal or microscopical agglutination test was equally unfruitful. Such results were not altogether surprising after considering the failure to obtain a satisfactory reading from precipitin-shake tests, in order to interpret the results as in the Kahn test.

The precipitate resulting from the precipitin reaction always remained in a more or less permanent state of suspension. In some instances, however, after standing for prolonged periods, precipitates disappeared altogether, as though they had been absorbed. It is possible that contamination and proteolytic enzymes might have played a part in such circumstances, or possibly the presence of an excess of antigen was responsible.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

II. SACCHARIFYING ACTIVITIES OF BARLEYS AND MALTS AND THE CORRELATIONS BETWEEN THEM[1]

By Henry R. Sallans[2] and J. Ansel Anderson[2]

Abstract

Determinations of free and total saccharifying activity were made on 144 samples of barley, and free saccharifying activity (Lintner value) was also determined on kilned malts made from these barleys. The samples represent 12 varieties of barley grown at 12 widely separated experimental stations in Canada.

Varietal differences were demonstrated with respect to each determination. In total barley activity and malt activity, Olli was outstandingly high; the remaining six-rowed rough-awned varieties, Pontiac, Mensury Ott. 60, O.A.C. 21, and Peatland, and the smooth-awned variety Velvet, also yielded comparatively high values; the two-rowed variety Hannchen gave intermediate values; and the two-rowed varieties Victory and Charlottetown 80, and the remaining smooth-awned six-rowed varieties, Nobarb, Wisconsin 38, and particularly Regal, were low in activity. With respect to free barley activity the varieties fell in the same order with the exception of Olli, Peatland and Charlottetown 80 which gave very low values. These three varieties have only about 22% of total barley amylase in free form whereas figures for the other nine varieties range between 38 and 44%.

There is a close correlation ($r = 0.997$) between total barley activity as measured by the papain and hydrogen sulphide methods, the former giving rather higher values. Varieties that are high in total barley activity also tend to be high in malt activity (papain, $r = 0.904$; H_2S, $r = 0.868$). A similar relation exists between free barley activity and malt activity for nine of the varieties ($r = 0.971$), but if the three varieties having low percentages of free amylase are included the correlation is not significant ($r = 0.217$). Environment affects each property in essentially the same manner so that mean values for the different stations fall in much the same order for each determination and correlation coefficients for station means are all high.

The possible utility of determinations of total barley saccharifying activity for facilitating the selection of strains of good malting quality from collections of hybrid lines is discussed.

As a result of investigations made by Myrbäck (14, and earlier papers) it appears to be well established that the amylase activities, both free and total, of barley and malt are varietal characteristics. Data accumulated by others (2, 5, 6, 9, 12, 17, 18) who have investigated amylase activity in barley or malt, or both, provide considerable additional support for this hypothesis. On the other hand, Chrzaszcz and Sawicki (8), in a recent paper based on an extensive investigation of the subject, take issue with Myrbäck and summarize their findings by writing "Ein Einfluss der Gerstensorte auf die Amylasemenge konnte nicht festgestellt werden." Myrbäck and Örtenblad have since replied in convincing manner (16).

The investigation described in this paper yields additional evidence on the effect of variety on amylase activity. It was undertaken with the object of obtaining information on varietal differences in barley and malt saccharifying

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[2] *Biochemist, National Research Laboratories, Ottawa.*

activities amongst a representative set of the barley varieties grown in Canada. The work also forms part of a more comprehensive investigation designed for the collection of an adequate body of data for the statistical examination of the relations between various barley and malt properties, and the bearing of these on the problem of evaluating the malting qualities of new varieties of barley.

Materials

The barley samples used in the present investigation were described in detail in Part I of this series of papers (1). Briefly, they consist of 144 samples representing 12 varieties grown at each of 12 widely separated experimental stations in Canada. The varieties are listed in Table I and the stations in Table II.

Duplicate malts were made from each barley sample in the malting laboratory at the University of Manitoba. The equipment and methods used have already been described (3). In order to control systematic errors that might arise in malting a series of samples that required three months to complete, the malting was carried out in the following manner. Each batch of malt contained 12 samples representing the 12 varieties grown at one station. The samples were arranged in random order within batches and the batches were malted in random order. The first replicates were made in the first 12 batches and the second replicates in the succeeding 12 batches. It will be apparent that these methods provide for an unbiased comparison of the varieties.

Methods

The free saccharifying activity of malt, *i.e.*, the Lintner value, was determined by the ferricyanide modification of the official method of the American Society of Brewing Chemists (4), and the results are reported in the usual manner in degrees Lintner. The saccharifying activities of the barleys were determined by the same method, with certain necessary modifications that are described below, and the results are also reported in degrees Lintner.

Barley samples were ground, shortly before use, in a Wiley mill with a 1.0 mm. sieve, and were then carefully mixed. Extractions were made at 20° C. using a 2.5-gm. aliquot of barley meal and 50 ml. of water contained in a 200–220 ml., wide-neck, volumetric sugar flask. The ratio of meal to solution was thus identical with that used for determinations made on malt.

Extractions for the determination of free saccharifying activity were made with distilled water for 2.5 hr.

In determining total activity by the papain method a 21.5-hr. extraction was made with the addition of 0.5 gm. of Merck's papain (*cf.* 10). For the determination of total activity by the hydrogen sulphide method, the flask was fitted with a rubber stopper and a delivery tube with a short length of rubber tubing and a pinch-cock. After introducing and mixing the barley meal and water, the air above the solution was displaced with hydrogen sulphide and the flask was allowed to stand for 20 min., with occasional shaking,

under a pressure of gas equivalent to 16 in. of water. This process was repeated and the flask, still under pressure, was then closed by means of a pinch-cock and allowed to stand in a water bath at 20° C. for 20.5 hr. At the end of this time the extracts were filtered, the air above the filtrates was displaced with hydrogen sulphide, and the flasks were stoppered until just before the solution was pipetted for the diastasis (*cf.* 7).

As a check on the precision of the analyses, duplicate determinations were made on one-third of the barley samples. The standard deviations of the means of duplicate determinations proved to be:— 0.9° L. for free activity; 1.7° L. for total activity by papain; and 1.9° L. for total activity by hydrogen sulphide.

Duplicate malts were made from each barley and single determinations of saccharifying activity were made on each malt. An estimate of the combined sampling, malting and analytical errors is therefore available with respect to this determination. The standard deviation of the mean of determinations made on duplicate malts proved to be 3.7° L.

Significance of Properties Measured

Before discussing the results of the investigation it seems wise to consider the significance of the various measurements that were made. In this matter there is room for considerable difference of opinion and the authors feel it incumbent upon them to state theirs.

Barley contains considerable quantities of the saccharogenic β-amylase. Only part of this can be extracted with water. According to Myrbäck and his co-workers (15, 16) the remainder is bound in some manner to the protein, and can be liberated by the action of the proteolytic enzyme papain, or of compounds, such as hydrogen sulphide, that stimulate the action of proteolytic enzymes already present in the barley. On the other hand, Chrzaszcz and his co-workers (7, 8, and papers cited therein) consider this hypothesis inadequate to explain all the data obtained by extracting barley in the presence of various enzyme preparations and enzyme-stimulating substances. They believe that barley contains sisto- and eleuto-substances, which also play a part in the reactions. It is generally agreed, however, that during the germination process most of the latent or inactive β-amylase is liberated or activated so that a very large proportion of the β-amylase present in malt can be extracted with water.

According to current hypotheses (13, p. 98), barley contains no free dextrogenic α-amylase, or at most, only small quantities of it. On the other hand, malt contains considerable quantities of α-amylase together with all the β-amylase originally present in the barley. Whether the α-amylase, or some essential activator for it, is actually elaborated during the germination process, or whether it is present in barley in unextractable or inactive form, remains a moot question. In malt both enzymes are present largely in such form that they can be extracted with water, and since α-amylase also has some saccharifying activity (11), determinations of saccharifying activity

made with aqueous extracts of malt measure the combined activities of free α- and β-amylase.

With these hypotheses in mind we may now consider what the determinations used in the present investigation actually measure. It is obvious, without further argument, that the determination of free barley saccharifying activity provides a comparative measure of the free β-amylase present in the samples.

Some differences of opinion may well exist with respect to the interpretation of the results of determinations of total barley saccharifying activity by the papain and hydrogen sulphide methods used in the investigation. Chrzaszcz and his co-workers (8, and papers cited therein) obtained higher values by extracting with a combination of papain and hydrogen sulphide than by extracting with either alone. They believe, however, that hydrogen sulphide has an activating effect on β-amylase activity. Myrbäck and Örtenblad (16) consider that hydrogen sulphide has very little stimulating effect and believe that the higher values obtained when hydrogen sulphide is used with papain are the result of activation of the papain by the gas. They stress the importance of using active preparations of papain and claim that with these the total amount of amylase present in the barley can be determined. The authors are of the opinion that the papain used in the present investigation was active. It gave higher values than hydrogen sulphide, whereas Chrzaszcz and Janicki (7) claim that higher values are obtained by the latter method. It seems probable that the papain method extracted all, or at least a very large proportion, of the β-amylase and thus provides a comparative measure of the total β-amylase contents of the samples. The hydrogen sulphide method, though it gave slightly lower results, placed the samples in almost exactly the same order as the papain method, and thus appears to provide an equally valid comparative measure of total β-amylase.

These conclusions are based in part on one other hypothesis that may be questioned, namely, that the papain and hydrogen sulphide methods do not extract any appreciable quantities of α-amylase if this enzyme is present in barley. Chrzaszcz's investigations show that extracts made by these methods have a more rapid action on starch-iodine color than extracts made with water alone. However, the change in color is of the type associated with the action of β-amylase, and the color is not completely destroyed in a comparatively short time as would happen if appreciable quantities of α-amylase were present in the extracts.

It appears that the determination of the free saccharifying activity (Lintner value) of kilned malt provides a comparative measure of the combined saccharifying activities of the free α- and β-amylase present in the samples, although the contribution made by the α-amylase is probably relatively small. Moreover, since data for 16 samples, published by Hills and Bailey (12), give a coefficient of correlation between free saccharifying activity of kilned malt and total saccharifying activity of green malt of 0.974, the present authors consider it probable that the former determination also

provides a fairly good comparative measure of the combined total saccharifying activities of the β-amylase present in barley and of the α-amylase that is elaborated or activated during malting.

Results and Discussion

Varietal Differences

The results of the investigation are summarized in Table I as means, over all stations, for each variety. Owing to the differential effect of environment on varieties, these did not all fall in the same order with respect to any determination at all stations. It was therefore necessary to resort to statistical analyses in order to determine whether the differences between varietal means could be considered significant. The results of the statistical analyses are given in a later section (Table III), but are summarized in the last line of Table I as necessary differences between means required for a 5% level of significance, *i.e.*, for odds of 19 to 1 that a real difference between varieties is operating to spread the means.

TABLE I

Mean saccharifying activities of malt and barley for each variety, in degrees Lintner

Variety	Class	Malt	Barley			
			Total		Free	
		Free, °L.	By papain, °L.	By hydrogen sulphide, °L.	°L.	Per cent of total by papain
A. *Olli*	6-row, rough awn	153	254	237	54	21
B. Pontiac	6-row, rough awn	131	227	209	96	42
C. Mensury, Ott. 60	6-row, rough awn	129	225	208	94	42
D. O.A.C. 21	6-row, rough awn	127	220	204	95	43
E. Velvet	6-row, smooth awn	124	217	202	97	45
F. Peatland	6-row, rough awn	120	246	231	51	21
G. Hannchen	2-row, rough awn	115	204	180	86	42
H. Victory	2-row, rough awn	103	188	167	82	44
I. Charlottetown 80	2-row, rough awn	100	204	192	46	23
J. Nobarb	6-row, smooth awn	100	197	182	75	38
K. Wisconsin 38	6-row, smooth awn	96	188	172	72	38
L. Regal	6-row, smooth awn	85	163	152	65	40
Necessary difference, 5% level		11	17	16	9	3

A comparison of the necessary differences with the actual differences between the means for the individual varieties leaves no room for doubt that each property measured is a varietal characteristic. The results of the investigation thus provide additional support for Myrbäck's conclusions (14).

The data for the saccharifying activity (Lintner value) of the malts, given in the first column of figures in Table I, show that the varieties are divided

into several groups. Olli has by far the highest activity; the rest of the six-rowed rough-awned varieties and the six-rowed smooth-awned variety Velvet form a group with fairly high activity; Hannchen yields an intermediate value; whereas the other two two-rowed varieties and the remaining three six-rowed smooth-awned varieties are low in activity, this being particularly true of Regal.

With respect to the total saccharifying activity of the barley, as measured by either the papain or hydrogen sulphide methods, the varieties fall in much the same rank order. The main discrepancies are caused by Peatland and Charlottetown 80, whose relative positions are improved.

These two varieties, together with Olli, yield anomalous results for free barley saccharifying activity. Whereas the other nine varieties fall in almost the same order with respect to the free saccharifying activities of both malt and barley, Olli, Peatland, and Charlottetown 80 yield very low values for free barley saccharifying activity.

The difference between the two groups of varieties is illustrated further by the data given in the last column, which give free barley activity as percentage of total barley activity (papain). Whereas Olli, Peatland, and Charlottetown 80 yield values of about 22%, the values for all other varieties fall between 38 and 44%.

Relations Between Properties Studied

Between Varietal Means

The more important relations between the various properties studied are illustrated by the scatter diagrams for varietal means shown in Fig. 1. Each point is represented by a circle containing a letter from which the variety can be identified by reference to the key given in the first column of Table I.

Fig. 1*A* shows the relation between malt saccharifying activity and total barley saccharifying activity as measured by the papain method, and Fig. 1*B* shows the corresponding relation for the values obtained by the hydrogen sulphide method. The correlation coefficients calculated from the data represented in these two diagrams were 0.904 and 0.868 (the remaining correlation coefficients obtained by the complete analyses of variance and covariance are given in Table IV).

It is apparent that there is a close relation between malt saccharifying activity and total barley activity and that those varieties that give high barley values also tend to give high malt values. This is the conclusion to which Myrbäck comes (14).

Looking at the matter from a different viewpoint, it also seems safe to assume that total β-amylase content, the property measured by the determination of total barley saccharifying activity, is the main factor controlling the comparative saccharifying activities of the malts made from different varieties. The data thus offer further support for the hypothesis advanced by Hills and Bailey (12), namely, that the β-amylase activity of malted grain can be predicted from the β-amylase activity of a papain digest of barley.

The data presented in Fig. 1*A* show, however, that β-amylase content is not the only factor governing the saccharifying activity of the malt though it may be, and probably is, the main one. If it were the only factor we might reasonably expect to find a much closer relation between total barley activity and malt activity, resulting in all the points in Fig. 1*A* falling much more nearly on a straight line.

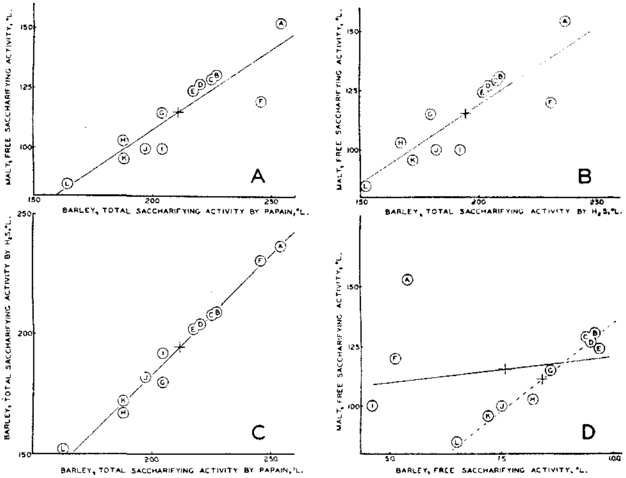

FIG. 1. *Scatter diagrams for varietal means showing relations between saccharifying activities of barleys and malts. The key for varieties is given in the first column of Table I.*

Among other controlling factors, α-amylase, with its contributing saccharifying activity, immediately suggests itself as important, and there are grounds for believing that the varieties differ in α-amylase content. Two possible situations must be considered. The varieties might well differ in α-amylase content, but in such a way that a very close correlation existed between α- and β-amylase contents: those varieties which were high in β-amylase content might also tend to be high in α-amylase content. If such a correlation existed and were close, it would not interfere with the relation between total barley saccharifying activity and malt activity. This situation may exist with respect to eight of the varieties studied. Reference to Fig. 1*A* will show that the points for all varieties except *F*, *I*, *J* and *K* lie almost exactly in a straight line.

The other situation in which the α- and β-amylase contents of varieties vary more or less independently must also be considered. Under these con-

ditions a reduction in the degree of associations between total barley saccharifying activity and malt activity would be expected, since those varieties that were comparatively low in α-amylase content would yield malts with lower activities than would be expected from consideration of comparative β-amylase contents only. In the light of these considerations there are grounds for believing that the varieties Peatland, Charlottetown 80, Nobarb, and Wisconsin 38, particularly the first pair, which are mainly responsible for reducing the correlation between total barley activity and malt activity (*e.g.*, points *F*, *I*, *J*, and *K* in Fig. 1*A*), are comparatively low in α-amylase content.

A difference in the response of varieties to the particular malting conditions used in the present investigation may also be responsible in whole, or in part, for the reduction of the correlation under discussion. Thus it may well be that the standardized set of malting conditions was unfavorable to Nobarb and Wisconsin 38, and particularly unfavorable to Peatland and Charlottetown 80, so that these four varieties failed to develop their potential saccharifying activity as fully as the remaining eight varieties.

Fig 1*C* shows the relation between total barley activity as measured by the papain method and by the hydrogen sulphide method. The correlation coefficient proved to be 0.995. A very close relation is thus demonstrated between the results of the two methods (*cf.* also Table IV), both of which placed the varieties in essentially the same order. It is interesting to note that the points *G*, *H* and *I*, which are furthest from the line, represent the three two-rowed varieties, Hannchen, Victory and Charlottetown 80.

Fig. 1*D* shows the relation between free barley saccharifying activity and malt activity. For nine of the varieties the relation is quite close, the correlation coefficient being 0.971. The other three varieties, Olli (A), Peatland (F) and Charlottetown 80 (I), which have only about 22% of the total barley amylase in free form, spoil the picture entirely and reduce the correlation coefficient to the insignificant value of 0.217. It is thus apparent that the hypothesis advanced by Shellenberger and Bailey (17), namely, that the diastatic activity of malt can be predicted by determining free barley activity, will not hold between all varieties, though data given in Table IV suggest that prediction is possible within individual varieties.

Between Station Means

Mean values for each station, over all varieties, are given in Table II. In order to facilitate comparison of the results of the various determinations, the stations are listed in order of descending values for malt saccharifying activity. Inspection of the data will show that the stations are placed in essentially the same order with respect to each determination. The close relations between malt activity on the one hand and the barley determinations on the other are shown more concisely by the correlation coefficients given in the last line of the table. All these are extremely high. It is thus apparent that most of the environmental factors that control one of the properties measured are also common to the control of the other properties.

The closeness of the relations between malt activity and barley activities is considerably reduced by the performance of the varieties at the first two and the fourth station. Malt values for Beaverlodge and Gilbert Plains are higher, and those for Lacombe are lower than would be expected from consideration of the barley data. It seems probable that these discrepancies are caused mainly by the two factors discussed in connection with between-varieties correlations, namely, differences in the proportions of α- and β-amylase produced under different environmental conditions, and a differential effect of malting conditions on samples from different stations.

TABLE II

MEAN SACCHARIFYING ACTIVITIES OF MALT AND BARLEY FOR EACH STATION, IN DEGREES LINTNER

Station	Malt	Barley			
		Total		Free	
	Free, °L.	By papain, °L.	By hydrogen sulphide, °L.	°L.	Per cent of total by papain
Beaverlodge	150	263	242	102	39
Lacombe	139	281	260	112	40
Ottawa	133	248	229	87	35
Gilbert Plains	133	262	243	88	34
Guelph	122	213	197	76	36
Ste. Anne de la Pocatière	121	218	201	74	34
Brandon	117	215	198	81	38
Lethbridge	116	216	199	83	38
Winnipeg	105	197	181	66	34
Ste. Anne de Bellevue	100	180	165	62	34
Fredericton	85	142	131	50	35
Nappan	63	99	89	33	33
Necessary difference, 5% level	11	17	16	9	3
Coefficient of correlation with malt values	—	.978	.977	.956	—

The last column of figures in Table II gives free barley saccharifying activity as percentage of total barley saccharifying activity by papain. The data show that with increasing total β-amylase content the percentage of free β-amylase remains roughly constant, ranging between 33 and 40%.

Statistical Analyses

The variance of the data for each determination was analyzed into portions due to (i) average differences between varieties; (ii) average differences between stations; and (iii) remainder. The last portion results not only from variations caused by a true interaction between stations and varieties, but also from variations caused by soil heterogeneity within stations, and by sampling and analytical errors. It therefore provides an adequate criterion for testing the significance of differences between station and varietal means.

The mean squares obtained by the analyses of variance are reported in Table III. Since the mean squares resulting from differences in the average performance of the individual varieties are significantly greater than the corresponding remainders, it is apparent that varietal differences exist with respect to each property determined.

TABLE III

ANALYSES OF VARIANCE FOR SACCHARIFYING ACTIVITIES : MEAN SQUARES

Variance due to	Degrees of freedom	Malt	Barley			
			Total		Free	
		Free	By papain	By hydrogen sulphide	Free	Per cent of total by papain
Varieties	11	4372**	7879**	7869**	4047**	1015**
Stations	11	7052**	33056**	28809**	5628**	58**
Remainder	121	166	425	376	120	12

NOTE: *In this and the following table* ** *denotes that the 1% level, and* * *that the 5% level of significance is attained.*

TABLE IV

ANALYSES OF VARIANCE AND COVARIANCE FOR SACCHARIFYING ACTIVITIES : CORRELATION COEFFICIENTS

Correlation between	Varieties	Stations	Remainder	Total
Total barley activity by hydrogen sulphide and total barley activity by papain	.995**	.999**	.985**	.997**
Malt activity and total barley activity by papain	.904**	.978**	.816**	.920**
Malt activity and total barley activity by hydrogen sulphide	.868**	.977**	.802**	.913**
Malt activity and free barley activity, *nine varieties only*	.971**	.982**	.781**	.945**
Malt activity and free barley activity, *twelve varieties*	.217	.956**	.649**	.658**
Free barley activity and total barley activity by papain	−.059	.974**	.667**	.652**

Certain pairs of sets of data were also subjected to analyses of variance and covariance. The resulting correlation coefficients are reported in Table IV. It is apparent that the correlation both within and between varieties is very close for total barley saccharifying activity by papain and by hydrogen sulphide, and fairly close for malt activity and total barley activity as measured by either method. For nine of the varieties there is also a correlation both within and between varieties for malt activity and free barley activity, but when all 12 varieties are studied together the inter-varietal correlation drops to an insignificant value. The inter-varietal correlation for

free and total barley activity is also insignificant, but a fairly close correlation apparently exists within varieties.

Applications to Barley Breeding

It appears that the measurement of β-amylase content in barley may prove to be a useful tool for plant breeders that are attempting to select from hybrid material those lines that are satisfactory from both the agronomic and malting viewpoints. The determination can be made quite rapidly (12 per day, per man) and requires only a few grams of grain and no expensive equipment. It should therefore be possible to apply it on a much wider scale, and at an earlier stage in the selections, than is possible with the laboratory malting test.

The possible utility of measurements of total barley saccharifying activity will depend upon whether there is a relation between the β-amylase content and the general malting quality of varieties. This may prove to be true if there are inter-varietal correlations between β-amylase and other hydrolytic enzymes, since it is generally agreed (*cf.* 13, p. 156) that in order to be *satisfactory for malting, varieties should be plentifully supplied with amylases,* proteases and hemicellulases. Unfortunately the literature on varietal differences in barley appears to contain no adequate body of data bearing on this important point. On the other hand, Hopkins and Krause (13, p. 146) whose opinions must be considered authoritative, think it reasonable to assume that a relation of this sort exists and that varieties that are well supplied with one type of hydrolytic enzyme will also tend to be well supplied with other types. With these considerations in mind, the present authors are inclined to think that the chance of selecting strains of satisfactory malting quality from hybrid lines that are high in β-amylase content will prove to be considerably greater than the chance of selecting such strains from hybrid lines that are low in β-amylase content.

It is apparent that it will be profitable to apply this selection method only to hybrid material resulting from a cross between a parent high in β-amylase content and of generally satisfactory malting quality, and a parent low in β-amylase content. Moreover, owing to the differential effect of environment on varieties, if the hybrid material is grown at one station only, the prevailing environmental conditions may tend to conceal average differences in β-amylase content which might be brought to light if the lines were grown at several stations. In spite of these limitations the selection method appears to be promising, though it is obvious that a good deal of further investigation will be required before its utility can be demonstrated.

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**In Table 1 of this paper (p. 382) the station names Ste. Anne de Bellevue and Ste. Anne de la Pocatiere should be transposed.*

HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

IV. FURTHER CROSSING RESULTS AND STUDIES ON THE F_1 HYBRIDS[1]

By L. P. V. Johnson[2]

Abstract

Results are given of hybridization work involving 27 *Triticum* forms and 18 *Agropyron* species. Only two *Agropyron* species, *A. glaucum* and *A. elongatum*, crossed successfully with *Triticum*. Several hundred F_1 plants have been obtained.

Hybrid seeds varied greatly in size; some were deficient in endosperm, others lacked the embryo. Seed germination and seedling nutrition were materially aided by use of 2 to 5% glucose solutions.

In general, *Agropyron* characters tended to be dominant in inheritance, particularly in crosses involving *A. elongatum*. The dominance relations for important characters are: *Agropyron* dominance in perenniality, vegetative vigor, and extent of mature root; partial *Agropyron* dominance in general morphological type, shattering of rachis, adherence of glumes to seeds, and winter hardiness; intermediate inheritance in texture of mature root, size of seed, rigidity of leaf, and leaf pubescence.

A method of root extraction is described in which plants are grown in special containers that permit reasonably normal root development and greatly facilitate extraction.

F_1 plants of crosses involving *A. glaucum* are completely sterile, while in *A. elongatum* crosses a fair proportion are moderately fertile. Chromosomal associations and relative proportions of functional and non-functional pollen are discussed in relation to fertility.

The more important results from similar work in Russia are outlined and discussed in relation to present results and future prospects of the investigation.

Introduction

Cross-pollination of *Triticum* with *Agropyron*, involving numerous species and varieties of each genus, was carried on extensively during the summers of the years 1935 to 1937. The first year's crossing results and descriptions of greenhouse-grown F_1 plants were reported by Armstrong (1). The present paper gives additional crossing results and F_1 data from both greenhouse and field obtained in 1936–1937, which brings this phase of the work up to date, prior to publication of data on later generations.

Materials and Methods

The *Triticum* materials used for crossing are listed in Table I under the heading "Female parents". Numbers 1 to 10 inclusive are winter varieties of *T. vulgare* (hexaploid, $2n = 42$); 11 and 12 are *T. vulgare*-type, true breeding, biennial wheat and rye hybrids; 13 to 19 are spring varieties of *T. vulgare*; 20 is a spring variety of *T. durum* (tetraploid, $2n = 28$); 21 is a spring form of the tetraploid species, *T. dicoccum*; 22 to 26 are miscellaneous tetraploid species; number 27, *T. monococcum*, is a diploid species ($2n = 14$).

[1] *Manuscript received August 6, 1938.*
Contribution from the Division of Forage Plants, Central Experimental Farm, Ottawa, Canada. This contribution forms a part of a co-operative investigation on the hybridization of Triticum and Agropyron, undertaken by the Dominion Experimental Farms Service and the National Research Council of Canada.

[2] *Formerly Research Assistant, Division of Forage Plants, Central Experimental Farm; now Forest Geneticist, National Research Council of Canada, Ottawa.*

Nearly all these species and varieties are fairly well known in North America. However, certain of the more recent introductions and productions will be described very briefly. Lutescens 0.62, Lutescens 0.329, and *T. timopheevi* were introduced from Russia, the latter being a variety with pubescent stem and leaves reputed to have a remarkable degree of resistance to pests and diseases generally. C.A.N. 1835 (R.L. 1005) is a high quality, rust-resistant variety developed at the Dominion Rust Laboratory from the cross Pentad × Marquis. Canus is a smut-resistant variety recently developed at the University of Alberta.

The readily-crossable *Agropyron* species (Table I) bear Division of Forage Plants numbers as follows: *A. glaucum* 1087, *A. elongatum* 820, 1083, 1300, 1301, 1302, and 1419. *A. glaucum* is hexaploid ($2n = 42$), *A. elongatum* is decaploid ($2n = 70$). These species, described in a previous report (1), are tall, vigorous, polymorphic, perennial bunch grasses having very extensive, fibrous root systems, coarse, harsh foliage, long spikes with shattering rachis, and fairly large seeds with lemma and palea adhering. *A. glaucum* is the shorter, less vigorous, softer in foliage, and more given to spreading by rhizomes. The strains used, especially those of *A. elongatum*, showed considerable variability.

The *Agropyron* species that did not cross successfully with *Triticum* are listed in Table II under the heading "Source of pollen". Division of Forage Plants numbers are given for each species.

Practically all crosses have been made using the *Triticum* parent as female. The reciprocal manner of crossing has the double disadvantage of necessitating emasculation of the stiff-glumed, smaller-flowered *Agropyron* and collection of pollen from the poorer pollen-yielding *Triticum*. It was found that percentage of seed set was much reduced when *Agropyron* was used as the female parent, which is in agreement with the findings of Russian investigators.

The crossing technique used in this work has been described in detail by Johnson and McLennan (5).

Crossing Results

The crossing results for 1936 and 1937 are given in Tables I and II. Table I summarizes the results of crosses involving *A. glaucum* and *A. elongatum*, the *Agropyron* species that cross readily with *Triticum*, while Table II summarizes the results from crossing attempts involving numerous *Agropyron* species that failed to cross successfully with *Triticum*.

In Table I the data from the six strains of *A. elongatum* have been bulked, since it is felt that variation in crossability and morphology within each strain approached the variation between strains.

It should be stated that in the cross Secalotricum × *A. glaucum*, 92 of the 93 seeds recorded in Table I were germless, and that the seeds recorded for *T. monococcum* × *A. glaucum* were also without embryos. Apart from these instances the seeds recorded were reasonably well-developed and potentially germinable.

TABLE I

Triticum-Agropyron CROSSING RESULTS FOR 1936 AND 1937. CROSSES INVOLVING READILY CROSSABLE *Agropyron* SPECIES

Female parents (*Triticum*)	Male parents (*Agropyron*)					
	A. glaucum 1087			*A. elongatum* (six strains)		
	Florets pollinated	Seeds obtained	Per cent success	Florets pollinated	Seeds obtained	Per cent success
1. Dawson's Golden Chaff	868	182	21.0	1,296	39	3.1
2. Minturki	885	32	3.6	425	19	4.5
3. Minhardi	608	99	16.3	–	–	–
4. Lutescens 0.329	1,596	7	0.4	–	–	–
5. Kharkov	665	227	34.1	1,008	14	1.4
6. White Odessa	1,170	173	14.8	–	–	–
7. Yaroslav	–	–	–	1,508	30	2.0
8. Kanred	–	–	–	691	9	1.3
9. Turkey Red	–	–	–	706	17	2.4
10. Crail Fife	–	–	–	1,316	0	0.0
11. Secalotricum	704	93	13.2	–	–	–
12. C.D. 1435	799	92	11.5	738	4	0.5
13. Lutescens 0.62	2,281	58	2.5	531	4	0.8
14. C.A.N. 1835 (R.L. 1005)	1,563	27	1.7	5,394	13	0.2
15. Marquis	902	18	2.0	364	3	0.8
16. Reward	361	26	7.2	–	–	–
17. Canus	664	30	4.5	273	0	0.0
18. C.A.N. 1352	–	–	–	86	2	2.3
19. Chinese	–	–	–	948	38	4.0
20. Mindum	1,228	73	5.9	484	39	8.1
21. Vernal emmer	220	14	6.4	130	0	0.0
22. *T. turgidum* 49	260	4	1.5	284	33	11.6
23. *T. turgidum* 131	348	1	0.3	276	6	2.2
24. *T. polonicum*	60	6	10.0	–	–	–
25. *T. persicum* var. Black Persian	240	20	8.3	94	1	1.1
26. *T. timopheevi*	–	–	–	562	23	4.1
27. *T. monococcum*	83	2	2.4	–	–	–
Total	15,505	1,184	7.6	17,114	294	1.7

Vernal emmer, which gave the highest percentage of crossability obtained with either *Agropyron* species in 1935, was more or less neglected subsequently since an abundance of hybrid seed was secured from the first year's work.

For the sake of simplicity, the *Triticum* material cross-pollinated by non-compatible *Agropyron* species is not itemized in Table II. In general, the results were essentially the same for all *Triticum* forms. An exception that is worthy of note is the disproportionately large number of germless "seeds" produced in crosses involving the wheat-rye hybrid, Secalotricum.

The production of germless seeds and stimulated ovaries from cross pollinations with *A. junceum*, *A. desertorum*, *A. imbricatum*, *A. sibericum*, *A. intermedium*, and *A. dasystachyum* may indicate that these species have relatively closer relations to *Triticum* than do the other non-compatible species of *Agropyron* listed in Table II. Stimulation of ovary development is not, however, considered to be the result of actual fertilization, but rather the result of chemical activation produced by the presence of partially germinated

TABLE II

Triticum-Agropyron CROSSING RESULTS FOR 1936 AND 1937. CROSSES INVOLVING NON-CROSSABLE *Agropyron* SPECIES

Source of pollen	Florets pollinated	Germless "seeds"		Stimulated ovaries	
		No.	%	No.	%
1. *A. junceum* 1086	4274	4	0.09	56	1.31
2. *A. desertorum* 1082	2234	2	0.09	0	0
3. *A. imbricatum* 995	1076	2	0.19	0	0
4. *A. sibericum* 1159, 1163, 1164, 1165	2890	0	0	2	0.07
5. *A. intermedium* 1085	3806	5	0.13	11	0.29
6. *A. dasystachyum* 931	1036	0	0	3	0.29
7. *A. cristatum* 653, 1081, 1158	3355	0	0	0	0
8. *A. cristatum* 655 (var. Fairway)	1549	0	0	0	0
9. *A. pauciflorum* 1267	640	0	0	0	0
10. *A. obtusiusculum* 1077	145	0	0	0	0
11. *A. Griffithsii* 1443	719	0	0	0	0
12. *A. Smithii* 73	530	0	0	0	0
13. *A. repens* 1076	40	0	0	0	0
14. *A. spicatum* 1060	210	0	0	0	0
15. *A. caninum* 1080	128	0	0	0	0
16. *A. Richardsonii* 932	40	0	0	0	0
Total	22,672	13	0.06	72	0.32

Agropyron pollen on the *Triticum* stigma. This interpretation suggests the possibility of relatively closer phylogenetic affinities of the species in question. Germless "seeds", however, are probably the result of partial fertilization, since there is a definite degree of endospermic development. It is believed that fertilization proceeded, in the latter, to the point where one of the sperm nuclei united with the secondary (or fusion) nucleus of the embryo sac to activate endospermic development, but that it did not proceed to the fusion of the second sperm nucleus with the egg nucleus (which would have initiated embryonic development). This presumes a much closer relation between species involved than mere stimulation of the ovary by chemical transmission.

In *T. persicum* var. Black Persian pollinated by *A. junceum*, one seed was produced which germinated and gave rise to a mature plant that was proved cytologically to be hybrid. Unfortunately, this plant died in transplanting.

Description of Hybrid Seeds

The effects of cross-pollination, in different and in the same parental combinations, ranged from slight to pronounced stimulation of the ovary, through intermediate gradations of endospermic without embryonic development, and varying degrees of endospermic with embryonic development, to the condition of a seed well developed in all respects (Plate I). Only seeds potentially germinable and with a reasonable amount of endosperm are considered in this section.

Hybrid seeds from different crosses exhibited great variation in shape and size which was closely correlated to the shape and size of seed in the respective

female (*Triticum*) parents. This is, of course, to be expected since *(a)* the $2n$ condition of maternal nuclear contribution to endospermic development would tend to emphasize maternal characteristics, *(b)* the seed coats are wholly maternal tissue and *(c)* the space conditions determined by the flowering glumes would tend to mold the shape and size of any seed developed therein.

A further variation related to hybrid seeds was the pronounced difference in germinability (or, more strictly speaking, the capacity for continued seedling development after the initiation of germination), the range being from 96.3% (Mindum × *A. glaucum*) to 11.9% (C.A.N. 1835 × *A. glaucum*). Such variation in germinability showed a close, direct correlation to seed weight. This correlation may be explained by assuming that the endospermic reserves in light seeds were insufficient to carry seedling nutrition to the point where rootlet absorption and photosynthesis take over the nutrition of the plant.

Cultural Methods

Germination of Hybrid Seed and Nutrition of Seedlings

The development of hybrid seedlings from seeds sown on or in sand and watered with tap water or a complete nutrient solution was often very poor, especially when seeds were markedly deficient in endosperm. Examination of such sowings revealed very frequent occurrence of seeds that had ceased development at an early stage. These observations led to experimentation designed to provide information on the nutrition of embryos in seeds deficient in endosperm. Since hybrid seeds were considered too valuable to subject to experimental risks, substitute material was prepared by excising (with small amount of endosperm retained) *Triticum* embryos (C.A.N. 1835), and by cutting the embryo ends (barely including the embryo) from seeds of *Agropyron elongatum* D.F.P. No. 1083.

Approximately 1000 gm. of sand was placed in each of 48 seven-inch, low-type, unglazed pots which were then allowed to stand in a shallow tray of water until thoroughly moistened. Twenty-five wheat embryos were sown to a depth of exactly $\frac{3}{8}$ in. in each of 24 pots, and the remaining 24 pots were similarly sown with clipped seeds of *Agropyron*. Four pots, two replications of each type of seed, were used to test each of the solutions used (Table III). Nowosad's *F* is a complete inorganic nutrient solution recommended for growing grasses and cereals in sand culture.

In order to observe directly the germinative behavior in the various solutions, 10 seeds from each material were sown on a blotter disc in a Petri dish, one dish to each of the 12 solutions. The discs were first moistened with tap water, then wetted with an excess of the solution.

After seeding, 24 cc. of the proper solution was added to each pot. The pots were flushed with 15 to 20 cc. of tap water about every second day (every day if evaporation was great). Six cc. of solution per pot was added by pipette each day for eight days, and thereafter on alternate days. The

guiding principle was to maintain approximately the original concentration of the solution, compensating for evaporation by the addition of water, for percolation and utilization by the addition of solution. Under the conditions of the experiment, efficiency in maintaining these conditions was entirely a matter of personal observation and judgment. The work was carried out under cool, damp, dull conditions in early winter. The experimental conditions could be improved greatly by the use of glazed pots and a humidity chamber.

Seedling emergence was general by the seventh day after sowing. The final seedling counts recorded in Table III were made on the fifteenth day.

TABLE III

DATA DEMONSTRATING THE EFFECTIVE NUTRITION PROVIDED TO EXCISED EMBRYOS OF *Triticum* AND *Agropyron* BY VARIOUS SOLUTIONS

Solution	No. of seedlings emerged, pots					No. of seeds germinated, Petri dishes			
	Triticum		*Agropyron*		Total out of 100	*Triticum*	*Agropyron*	Total out of 20	%
	1	2	1	2					
Tap water	0	1	5	4	10	5	8	13	65
Nowosad's F	0	0	3	4	7	4	5	9	45
Glucose 0.5%	3	1	9	11	24	6	7	13	65
Glucose 1.0%	0	2	8	13	23	7	8	15	75
Glucose 2.0%	3	4	13	13	33	7	8	15	75
Glucose 3.0%	10	9	13	19	51	7	7	14	70
Glucose 5.0%	8	13	18	17	56	8	8	16	80
Maltose 0.5%	0	0	10	10	20	8	7	15	75
Maltose 1.0%	1	0	9	6	16	7	7	14	70
Maltose 2.0%	1	1	5	4	11	7	9	16	80
Maltose 3.0%	0	0	6	4	10	6	10	16	80
Maltose 5.0%	0	1	5	6	12	5	8	13	65

The emergence data in Table III demonstrate that glucose solutions in all concentrations give a marked increase over the control, while the germination (direct observation) data indicate only a slight increase from these solutions. This may be explained by assuming that about 65% of the embryos were capable of germination but that sugar nutrient was required to enable the seedlings to emerge through $\frac{3}{8}$ in. of sand. Maltose solutions appear to have stimulated germination, but were indifferent in emergence tests. The latter result may have been due to the definite crusting of the sand at the surface with maltose solutions of 1% or over. The complete nutrient solution (Nowosad's *F*) was detrimental to germination. This type of nutrition is, apparently, effective only after rootlet absorption and photosynthetic activity have become established.

At this stage of the experiment the pots were thoroughly flushed and the plants grown for several weeks with complete nutrient solutions (Nowosad's *F* and others).

Two per cent glucose solution was applied (flushed alternately with water) to several hundred hybrid seeds sown in sand. As a control, one of two trays

of comparable material was treated with glucose, while the other received water only. Emergence from the tray treated with glucose was 128 seedlings from 148 seeds (86.5%), while only 81 seedlings were obtained from 125 seeds (64.8%) in the tray receiving water only.

Growing of F_1 Plants

Since the F_1 plants were perennial, and would therefore normally flower for the first time in the second year of their existence, and since it was desirable to induce flowering in the greenhouse during the first winter, special treatment was required. The hybrid seeds were collected from cross-pollinated *Triticum* plants in August and sown in sand in greenhouse flats in late September. About two weeks later the seedlings were transplanted to soil in 2½-in. pots in which they remained, under the coolest possible greenhouse conditions, for several weeks. During this time the leaves were cut back successively and advantage taken of opportunities to place the plants under cool outdoor conditions and gradually to subject them to mild freezing. This treatment simulated the winter conditions which biennial and perennial plants normally require before flowering. About the middle of November the plants were placed under optimum growing conditions for about a week and then transplanted to 5-in. pots, where they remained until maturity. Early in January the period of artificial illumination was increased so that total daily illumination was 17 to 18 hr. Flowering commenced early in February and, in the fertile plants, seed was matured by late March. The plants were then cut back and superior individuals cloned and repotted. Plants were placed out of doors early in May and transplanted to the field about two weeks later.

Description of F_1 Plants

Seedling Characters

Observations on number of rootlets on germinated seeds of parents and hybrids may be summarized as follows: spring wheats usually have five or six rootlets, maximum nine; winter wheats tend to have a lesser number; *A. glaucum* usually has two or three rootlets, *A. elongatum* one or two. These parental characteristics are reflected in hybrid seedlings. Hybrids between spring wheats and *A. glaucum* tend to average three or more rootlets, while hybrids of spring wheats and *A. elongatum* tend to average less than three; when winter wheats are involved, these averages tend to be less. In rootlet number the *Agropyron* parents exert a dominant heritable influence which is, however, not quite complete, especially in *A. elongatum*. Most of these points substantiate the data given in Table VI of Armstrong's report (1).

Color of the coleoptile was studied in parental and hybrid material. The coleoptile of *A. glaucum* and *A. elongatum* is purplish, while in most wheats it is green. In the hybrids, coleoptile color tended to be like that of the *Agropyron* parents.

Observations on juvenile posture (angle between leaves and the ground), made at about the five-leaves stage, reveal a marked tendency for the semi-prostrate condition of the *Agropyron* parent to be transmitted to the hybrid.

It is hoped that correlations between seedling characters and important mature-plant characters in segregating generations may prove useful in the selection of superior types at the seedling stage.

General Upper-plant Characters

The previous report by Armstrong (1) gave general and detailed descriptions of the F_1 hybrids and parental types grown in the greenhouse. The present descriptions are more detailed and are based on more extensive observations on both greenhouse and field-grown plants. They may be considered as being supplementary to the previous descriptions.

During the late winter of 1936, detailed observations were made on greenhouse material, both hybrid and parental, at the time of flowering or shortly thereafter. The data from these observations are given in summarized form in Tables IV and V. In summarizing, the data compiled from each of several plants studied in a given material were arithmetically or otherwise averaged for each separate character. In this way a single term was obtained which indicates the average or typical expression of the character in question.

Table IV presents summarized descriptions of spike and culm characters, while Table V deals similarly with leaf characters.

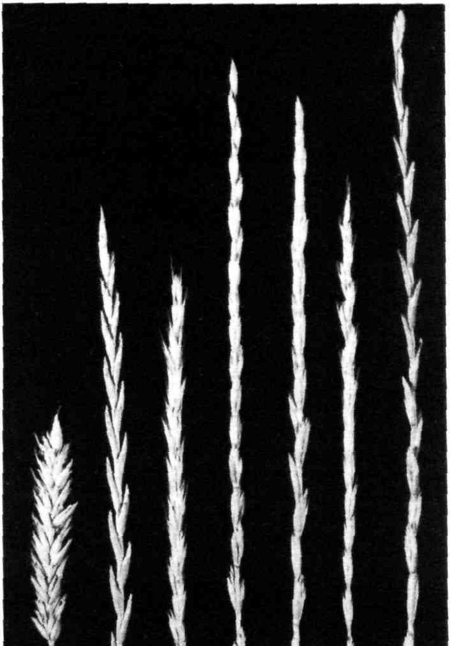
The data in Tables IV and V will be discussed in connection with Table VII, which serves further to summarize these data as they relate generally to the phenomena of dominance, transgressive expression and hybrid vigor.

After growing to maturity in the greenhouse, the hybrid materials (upon which data in Tables IV and V are based) were cloned and transplanted to the field in the spring of 1936. Field observations made throughout the summer are summarized in Table VI.

In the field the hybrid material, with one exception, grew very vigorously. The exception was the Mindum × *A. glaucum* material, in which about 90% of the plants gradually died or formed a few, weakly erect or decumbent stems. In the case of vigorous hybrids of this cross, all individuals of the clone were vigorous, and the same clonal uniformity was true with respect to non-vigorous hybrids. Beyond all reasonable doubt, therefore, the developmental response was genetic rather than purely environmental.

In the first year's field growth, the occurrence of tufted plants was particularly prevalent in Kharkov × *A. glaucum*, C.A.N. 1835 × *A. glaucum*, and Vernal emmer × *A. elongatum* materials. This response appeared to be related in most crosses to perenniality, while in others, notably Kharkov × *A. glaucum*, it appeared to be due to developmental disturbances probably related to hybridity. In every instance the response was uniform among the individuals of the clone, indicating a genetic rather than a purely environmental basis.

The susceptibility to ergot found in *A. glaucum* crosses is, in all probability, inherited from *A. glaucum*, which is fairly susceptible. The degree of susceptibility shown by *A. glaucum* hybrids involving Kharkov, Lutescens 0.62, and Vernal emmer is, however, considerably greater than has been observed



Mature spikes of: 1. *Triticum vulgare* var. *Lutescens 0.62.* 2. *Lutescens 0.62* × *A. glaucum.* 3. *C.A.N. 1835* (*T. vulgare, bearded*) × *A. glaucum.* 4. *Lutescens 0.62* × *A. elongatum.* 5. *C.A.N. 1835* × *A. elongatum.* 6. *A. glaucum.* 7. *A. elongatum.*

TABLE IV

SUMMARIZED DETAILED DESCRIPTIONS OF SPIKE AND CULM CHARACTERS TYPIFYING CERTAIN PARENTS AND HYBRIDS GROWN IN THE GREENHOUSE

Material	No. of plants	Spike							Culm				
		Density index	No. of spikelets	No. of florets	Awning	Secondary glume			Plant height, in.	No. of nodes	Pubesc. of node	Diam. culm, mm.	Diam. neck, mm.
						Shoulder	Beak	Keel					
A. glaucum	5	89.0	18.8	3–4	1	N	M, M-A	M	50.6	4.0	0	1.79	1.10
A. elongatum	10	139.8	14.24	5.7	0	N-M	0	Wk-M	54.9	3.4	0	2.21	1.37
Lutescens 0.62	5	58	16.2	4	0–1	W	S, B	St	38.8	3.0	+	3.19	1.79
Lutescens 0.62 × *A. glaucum*	12	,67.6	19.8	3–4	0–1	N	S, A	St	66.0	4.3	0	2.41	1.41
Lutescens 0.62 × *A. elongatum*	10	100.5	15.7	4–6	0	M	0–S, A	Wk–M	63.7	3.5	0	2.35	1.39
C.A.N. 1835	6	49.3	23.9	4–5	3	M	L, A	M	53.3	4.2	+	3.64	2.48
C.A.N. 1835 × *A. glaucum*	8	68.4	17.7	3–4	0–1	N	S–M, A	St	50.5	4.3	0	2.16	1.27
C.A.N. 1835 × *A. elongatum*	10	118.9	13.6	4–6	0	M	0	Wk	59.1	3.2	0	2.29	1.34
Kharkov	5	47.1	26.0	4	3	W	L, A	St	54.8	4.8	Tr	3.30	2.00
Kharkov × *A. glaucum*	11	72.0	20.1	3–5	0–2	N–M	S-L, A	St	53.5	4.4	0	2.47	1.52
Kharkov × *A. elongatum*	17	108.0	14.1	4–6	0–Tr	M–W	0–S, A	M	58.6	3.4	0	2.26	1.28
Mindum	5	34.2	30.6	3–4	3	N	L, A	St	55.2	4.7	0	4.62	1.26
Mindum × *A. glaucum*	10	59.5	17.9	3–4	1–2	N	S–M, A	St	59.1	4.3	0	2.13	1.07
Mindum × *A. elongatum*	4	101.0	11.0	3–4	0–Tr	N	0–Tr	Wk–M	54.0	3.9	0	1.69	0.98
Vernal emmer	5	30.3	24.2	3–4	3	N	S, B–A	St	56.4	4.3	+	2.71	1.29
Vernal emmer × *A. glaucum*	10	54.0	22.0	3	1–2	N	S, A	St	59.8	4.2	0	2.26	1.17
Vernal emmer × *A. elongatum*	10	95.8	11.2	4–6	0	M	0–S, A	M	59.6	4.4	0	1.66	0.97

EXPLANATION: Spike density index refers to the distance in mm. along the spike occupied by ten typical spikelets, thus the larger the index the smaller the density. Under awning, 0 denotes awnless, 1 short awns, 2 medium awns, etc. The secondary glume is the outer glume that originates slightly above the opposing outer glume; it is very constant in expressing varietal differences. Under secondary glume beak, the symbol preceding the comma refers to length of beak, while the symbol following the comma refers to sharpness of beak. Culms were measured by micrometer about 2 in. above second topmost node, necks were similarly measured about an inch below spike.

SYMBOLS: 0—absent; Tr—trace; +—present; N—narrow; M—medium; A—acute; B—blunt; S—short; L—long; Wk—weak; St—strong.

TABLE V

SUMMARIZED DETAILED DESCRIPTIONS OF LEAF CHARACTERS TYPIFYING CERTAIN PARENTS AND HYBRIDS GROWN IN THE GREENHOUSE

Material	No. of plants	Leaf											
		Length, cm.	Width, mm.	Pubescence			Venation			Marginal barbing	Rigidity	Auricle color	Ligule length
				Dorsal	Ventral	Marginal	No.	Barbing					
								Dorsal	Ventral				
A. glaucum	5	17.6	7.5	0–S	M–L	0–¾L	7.4	0–M	St	St	M	Wh	S–M
A. elongatum	10	17.0	4.9	0	0–M	0–BM	5	Wk	St	M–St	St	Wh	S–M
Lutescens 0.62	5	26.4	14.4	S	S	BS	10.6	0	+	M	Wk	Wh	M
Lutescens 0.62 × *A. glaucum*	12	20.2	9.9	S	M	CS–¾M	8.2	Wk	+	St	Wk–M	Wh	S–M
Lutescens 0.62 × *A. elongatum*	10	19.3	7.5	0–S	S–M	0–BS	7.5	Wk	M	M–St	M	Wh	S
C.A.N. 1835	6	31.4	17.7	S	S	CS	11.8	Wk	+	M	Wk–M	Wh	M
C.A.N. 1835 × *A. glaucum*	8	13.0	9.5	0–S	0–S	0–CM	8.2	0	+	Wk–M	Wk–M	Wh	S–M
C.A.N. 1835 × *A. elongatum*	10	21.0	7.3	0–S	S–M	0–BS	7.2	0–St	Wk–St	M–St	M–St	Wh	S
Kharkov	5	18.1	12.6	0	0	CM	9.6	+	M	Wk	M	P	L
Kharkov × *A. glaucum*	11	16.9	10.9	0	0–S	0–BS	8.4	0–M	+	Wk–M	M	P	S
Kharkov × *A. elongatum*	17	21.1	7.2	0	0–M	0–½M	7.0	0	St	St	M–St	P	M
Mindum	5	18.5	12.5	0	0	CS	9.2	Wk	+	Wk	Wk	Wh	M–L
Mindum × *A. glaucum*	10	16.1	8.2	0	0–S	0–BS	7.7	0–Wk	+	M	Wk–M	Wh	M
Mindum × *A. elongatum*	4	15.3	4.7	0	0–S	0–BS	6.9	Wk	St	M	M–St	Wh	S
Vernal emmer	5	22.2	12.0	Tr	S	BS	9.3	0	0	Wk	Wk	Wh	L
Vernal emmer × *A. glaucum*	10	17.6	9.7	0–Tr	S–L	CS–¾M	7.9	0	0	M–St	Wk–M	Wh	M
Vernal emmer × *A. elongatum*	10	16.6	5.7	0–S	S–M	0–CS	7.0	Wk	M	M	M	Wh	S–M

EXPLANATION: Highest typical leaf used, usually topmost, occasionally second topmost. Under pubescence marginal, combinations of symbols are to be read as follows: 0–¾ L denotes a range from absence to long hairs for three-quarters of length of leaf, BS denotes short hairs at base of leaf, MC denotes medium length hairs at colar of leaf, etc.

SYMBOLS: 0—absent; Tr—trace; +—present; S—short; M—medium; L—long; ½, ¾—portion of leaf affected; B—base; C—colar; St—strong; Wk—weak; Wh—white; P—purple.

TABLE VI

SUMMARIZED GENERAL DESCRIPTIONS OF CERTAIN HYBRIDS GROWN IN THE FIELD

Cross	No. of plants	Days to *heading*	Pos-*ture*	Relative height	Relative no. of culms	Leafi-ness	*Stem* (solid or hollow)	Reaction to *Ergot*	Reaction to Leaf rust	Forage quality	Remarks
Mindum × *A. glaucum*	52	41-98, 57	1-4, 2	1-3, 2	1-3, 1	1-2, 1	S-H, S-H	0-2, 1	1 2, 1	Poor-fair; short, rather harsh leaves.	4 plants of fair-good forage quality.
Kharkov × *A. glaucum*	35	45-96, 61	2-3, 2	1-3, 3	1-4, 3	1-3, 2	S-H, H	1-4, 3	0-3, 2	Poor-fair; medium long, rather harsh leaves.	2 plants of fair-good, many tufts.
Lutescens 0.62 × *A. glaucum*	21	45-54, 49	2-3, 3	2-3, 3	3-4, 4	3 3	S-H, S	1-4, 3	1-2, 1	Poor-fair; long, rather harsh leaves.	Very uniform.
C.A.N. 1835 × *A. glaucum*	7	45-60, 52	2-3, 2	1-3, 2	1-4, 3	1-3, 2	S-H, S	1-2, 1	1-2, 1	Poor-fair; short, rather harsh leaves.	Many tufts.
Vernal emmer × *A. glaucum*	53	45-84, 57	2-4, 3	2-3, 3	3-4, 4	3-4, 4	S-H, S	0-3, 2	0-2, 1	Poor-fair; medium long, rather harsh leaves.	
Mindum × *A. elongatum*	6	56-84, 71	3-4, 4	2-3, 3	2-4, 3	3, 3	S-H, S-H	0	1, 1	Poor-fair; narrow, rather harsh leaves.	Considerable variation.
Kharkov × *A. elongatum*	38	41-68, 52	2-3, 2	2-4, 3	3-4, 3	2-3, 2	S-H, S-H	0	1-2, 1	Poor; stiff, rather harsh leaves.	
Lutescens 0.62 × *A. elongatum.*	16	43-59, 47	2-3, 2	2-4, 3	2-4, 4	2-3, 2	S-H, S-H	0	1-2, 1	Poor; stiff, rather harsh leaves.	
C.A.N. 1835 × *A. elongatum*	13	45-63, 51	2-3, 2	3, 3	3-4, 4	2-3, 2	S-H, S-H	0	1-2, 1	Poor; stiff, rather harsh leaves.	
Vernal emmer × *A. elongatum.*	51	55-102, 75	3-4, 3	3-4, 3	3-4, 4	2-3, 2	S-H, S-H	0	0-2, 1	Poor; stiff, rather harsh leaves.	Many tufts.

EXPLANATION: Numerical increase denotes quantitative increase in character expression: thus, 1 denotes short, 2 medium short, 4 tall, etc. In the case of posture, 1 denotes prostrate, 4 denotes erect. In each column the data preceding the comma refers to the range of expression, while the data following the comma refers to the average or typical expression.

in *A. glaucum*. The wheats, parental in these instances, have not been observed to be particularly susceptible to ergot. The characteristic ergot immunity of *A. elongatum* is reflected in its hybrids.

Quality of forage varied greatly with age and position of the leaves. New leaves, and tufted leaves at the base of the plant, were relatively much softer than older or upper leaves. In general, the *A. glaucum* hybrids have the softer foliage, the best in this respect being Mindum × *A. glaucum*. Unfortunately, this hybrid is usually non-vigorous. Among *A. elongatum* crosses, Mindum also gave the softest foliage, combined with excellent vigor. Among the *Triticum* parents, Mindum has noticeably the softest leaves.

The vegetative period in all hybrids, but particularly in those of *A. elongatum*, is prolonged into late fall. The degree of expression in this regard exceeds the limits of either the *Triticum* or *Agropyron* parents (transgressive expression) and appears, with exception of *A. glaucum* crosses with Mindum and C.A.N. 1835, to be related to hybrid vigor.

The winter hardiness of the hybrids was severely tested during the winter of 1936–37 when the amount of winter killing among perennial crops was extremely high. In general, it may be said that the *Triticum-Agropyron* hybrids survived about as well as native *Agropyrons*. Both *A. glaucum* and *A. elongatum* hybrids involving Kharkov, the only winter-wheat parent at the time, survived much better than any other. This observation led to the emphasizing of winter wheats as *Triticum* parents in subsequent crossing. The winter of 1937–1938 was not sufficiently severe to bring out differential reactions to freezing.

Hybrids of *A. glaucum* have a moderate tendency to spread by short rhizomes, a characteristic inherited from *A. glaucum*. The *A. elongatum* hybrids, like their *Agropyron* parent, have not rhizomes, but by profuse tillering attain large, bushy proportions at maturity.

Perenniality appears to be completely dominant in all F_1 hybrids. It remains to be seen, however, whether the hybrids will live as long as their *Agropyron* parents.

Leaf Characters Related to Drought Resistance and Palatability

Since drought resistance and forage palatability are both primary considerations in the practical objectives of the work, leaf characters related to these qualities are very important. Preliminary studies of a comparative nature have been made on cross sections and stomatal surfaces of the leaves of the F_1 plants and parents of the more important crosses (those dealt with in Tables IV and V).

Leaf material for cross sections was collected from field-grown plants immediately after flowering, central portions of typical upper leaves being taken. A 6% solution of formalin proved to be superior as a killing and fixing agent to Navashin's or alcoholic agents, since it caused less hardening and facilitated cutting, which was done with a hand microtome using pith imbedding. Sections were cut at approximately 50 μ, fixed on slides with

Szombathy's agent (4), stained by modifications of Flemming's safranin, gentian-violet and orange G, or Chamberlain's safranin and light green schedules (4) and mounted in balsam. Differential staining was good with either schedule, with preference for the triple stain. After preliminary examinations of stained sections, general comparative observations were made on freshly cut, unstained temporary mounts.

Material (either fixed or fresh) for stomatal work was prepared by placing downward the epidermis to be studied and scraping away all other tissues with a razor blade. Excellent differential staining was obtained with safranin (1 gm. in 150 cc. of 50% ethyl alcohol).

Observations on cross sections of the leaves of parents and hybrids indicated a very similar relation between parents and hybrids in all crosses. This permits a general discussion of observations with only occasional reference to specific materials.

In cross section, the upper surface of the leaf of *A. elongatum* appears as a continuous series of alternately high and low, steep-sided, round-topped undulations (ridges and furrows). Bulliform (motor) cells occur at the base of the ridges, and stomata occur in a staggered row on either side of the ridge. Girder tissue of thick-walled, sclerenchymatous cells extends on either side of the vascular bundle, with increasing width, to the epidermis. The lower surface of the leaf is non-ridged with stomata between girder outcroppings. These general characters are transmitted with a few minor differences to *Triticum* × *A. elongatum* hybrids. Influence of *Triticum*, which is only slightly ridged, is expressed in the hybrids as a marked flattening of the tops of the ridges. While the amount of girder tissue in the hybrids is about the same as in *A. elongatum*, the cell walls of the hybrid tissue are much thinner, being intermediate between the parents. This probably accounts for the intermediacy of leaf rigidity in the hybrids (Tables V and VII).

Thickness of cuticle of the upper epidermis is much greater in *A. elongatum* than in *Triticum*, and this character is to a considerable degree transmitted to the hybrids. The cuticle of the lower epidermis is thick in *A. elongatum* and moderately thick in *Triticum*. This lack of clear-cut difference makes classification of the hybrids difficult, but they are believed to have a slightly thicker cuticle than the *Triticum* parents.

The differences between *A. glaucum* and *Triticum* for most of the characters discussed above are not very pronounced. While the genetic transmission of characters to the hybrids is not easily observed, it is considered to be essentially the same as for *A. elongatum* hybrids. The amount of girder tissue in hybrids reflects the greater amount present in the *A. glaucum* parent, but cell-wall thickness in the hybrids is considerably thinner owing to a *Triticum* influence. The flattening of the top of ridges in the hybrids, mentioned above, is also noticeable in *A. glaucum* hybrids.

A. elongatum has a pronounced, continuous, parenchymatous sheath surrounding the vascular bundle, while in *A. glaucum* and the *Triticum* parents this sheath tends to be less pronounced and somewhat discontinuous.

Triticum × *A. elongatum* hybrids appear to be identical with their *Agropyron* parent in this character.

Stomatal counts proved to be so variable that significant differences could not be established.

A survey of some of the literature dealing with xeromorphic characters reveals a wide difference in the emphasis placed by various workers upon the importance of these characters to drought resistance. In the present paper this question is left entirely open. There are several so-called xeromorphic characters represented in the leaves of the *Agropyron* parents, especially *A. elongatum*. These may be listed as follows: ridging of leaves, bulliform cells, and presence of stomata in leaf furrows, considered by some to be jointly related to drought resistance in connection with leaf rolling; sclerenchymatous tissues and parenchymatous sheaths surrounding vascular bundles as a possible protection against excessive water loss through spongy tissues; relatively large proportions of sclerenchyma as compared with parenchyma; and heavy cuticular development. These characters tend to be transmitted to the hybrids.

It is important to note, however, that many of these characters, considered favorable from the point of view of xerophytism, are extremely unfavorable from the point of view of forage palatability. It may be necessary, therefore, in subsequent breeding work, to effect a compromise between xeromorphism and forage quality.

More complete histological data, discussed from the viewpoint of xerophytism, are given by McLennan (6).

Root Characters

Comparative studies have been begun on the root systems of parents and hybrids in order to provide practical information that, together with that derived from histological studies of leaves, might contribute toward a better understanding of the morphological bases of drought resistance.

The great obstacle in root studies is the extremely laborious task of root extraction. A special container in which the roots are grown was devised which affords an approach to the ideal of maximum reduction in time and labor of extraction with minimum reduction in quality of root. In its unassembled condition the container consists of two pieces of galvanized sheet metal each bent to form a right-angular, V-shaped trough. Along one of the straight edges of each section, the metal is bent to make a groove about an inch deep which will admit the unbent straight edge of the other. In assembling, the two sections are held in place by small bolts inserted at four points along the connection. The assembled container is a box-like object 36 in. high, 16 in. square and open at both ends.

Forty-nine containers were placed in seven equal rows, slightly sunken in cultivated soil and banked with soil to the top edges of the outermost containers. In filling the containers, an effort was made to simulate natural soil horizons and care was taken to pack the soil thoroughly and evenly at

the time of filling. If possible, the soil should be prepared one year in advance, particularly in dealing with annual plants. Plants are started from seed or from very small, uninjured seedlings.

When a given root system is to be extracted, the container with plant and soil intact is removed to a tank of water, laid on its side and soaked overnight, after which the upper section of the container is removed and, beginning at the root end, extraction is made by the Pavlychenko spray method (7). The container proved to be near the maximum size that could be handled without special means for lifting and moving.

This method was quite satisfactory for extensive perennial roots, especially if two or more years old. Difficulty with breaking of the soil block was experienced in moving containers in which annuals were growing in soil which had been filled in only a few months previously. This difficulty was largely overcome by permitting soil to settle in the container one year before use.

The root systems obtained by this method are not complete, nor can they be considered strictly typical of field-grown roots. Nevertheless, they are considered to be sufficiently similar to normal roots in type and extent to permit evaluation for genetic and breeding purposes.

During the summer of 1937 some 20 roots were extracted, of which 15 were preserved in 4% aqueous formalin solution for later detailed studies. These roots are as follows:

C.A.N. 1835	
Kharkov	(two roots)
A. elongatum 820	(one second-year root)
A. elongatum 1083	(two second-year roots)
A. glaucum	(two second-year roots)
Kharkov × *A. elongatum* 1083, F_1	(two second-year roots)
Vernal emmer × *A. elongatum* 820, F_1	(two second-year roots)
Vernal emmer × *A. glaucum*, F_1	(two second-year roots)
Lutescens 0.62 × *A. elongatum* 1083, F_1	(one second-year root)

Preliminary observations on roots of the *Agropyron* parents reveal very extensive systems with large numbers of main branches which tend to be coarse, especially in *A. elongatum*. The wheat parents, especially spring wheats, have smaller root systems with branches of much finer texture. The root systems in the F_1 hybrids are fully as extensive as those of the *Agropyron* parents and show a pronounced tendency toward the fine-textured branches of the *Triticum* parents (Plate II). This is considered to be a very favorable combination of parental characteristics.

It is questionable whether the occurrence of very extensive root systems in F_1 plants is due to dominance of the *Agropyron*-type root or to hybrid vigor. Observations in F_2 roots should provide an answer to this question.

These preliminary observations on F_1 hybrid root systems indicate the probable occurrence of a preponderance of superior roots among hybrid segregates. There is no reason at present, therefore, to doubt the possibility

of selecting hybrid plants having root systems that will provide resistance to drought and that will have good soil-binding properties.

Summary of Descriptions of F_1 Plants

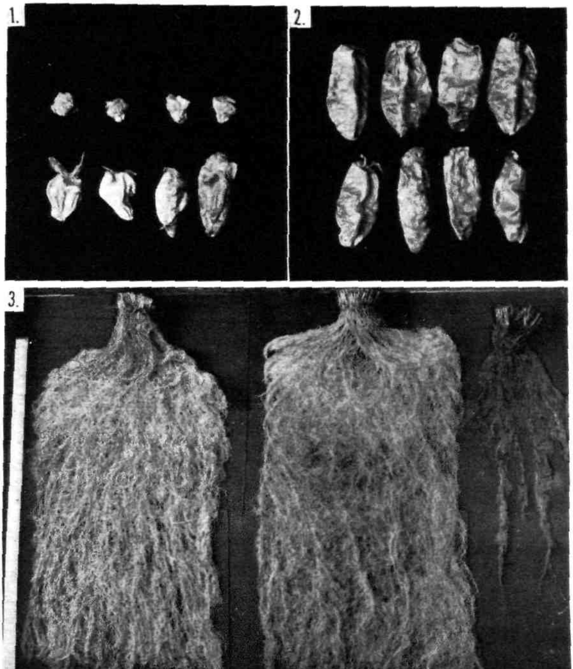
To facilitate general summarization the descriptive data compiled from the original, and more thoroughly studied, F_1 hybrids have been brought together in Table VII in which they are examined as a whole, on the basis of relative genetic dominance, transgressive expression, and hybrid vigor (compare Table VII of Armstrong's report (1)).

The symbols in Table VII are used as follows: *A* and *T* denote apparently complete dominance of the ***Agropyron*** and ***Triticum*** conditions, respectively; *I*, *IA* and *IT* denote intermediate, intermediate-***Agropyron*** and intermediate-*Triticum* conditions, respectively; *X*, *XA* and *XT* denote indeterminate transgressive expression, and transgressive expressions transcending ***Agropyron*** and *Triticum* parents, respectively. The term "transgressive expression" denotes a degree of expression that goes beyond the limits observed in the parents; the degree of expression may be lower than the low-degree parent, higher than the high-degree parent, or it may be indeterminate, *i.e.*, not definitely related to the type of expression found in either parent. The dashes indicate lack of character differential in the parents, lack of expression in hybrids, or lack of data.

Certain characters included in Table VII but not discussed previously, and concerning which there may be some vagueness, will be discussed briefly. The term "life tenure" refers to the condition of being annual, perennial, etc. The perennial ***Agropyron*** condition appears to be completely dominant. The "heading-to-flowering" period (time from spike emergence to anthesis) differs widely in the parents, being for *Triticum* usually less than a week, for ***Agropyron*** two or more weeks, and for the hybrids the same as for ***Agropyron*** (or slightly less). "Posture" refers to the condition of being erect, prostrate, etc. In juvenile posture (five-leaves, seedling stage) ***Agropyrons*** and winter wheats tend to be prostrate, under cool conditions, while spring wheats tend to be erect; the hybrids approach the ***Agropyron*** condition. At maturity the ***Agropyron*** stems are somewhat spreading (mature posture) while ***Triticum*** stems are erect; with the exception of Mindum × ***A. elongatum*** which tends to be more erect, the hybrids closely approach the ***Agropyron*** condition.

The summation of frequencies of the various expressions indicates a dominant or partially dominant influence by the ***Agropyron*** parent. Hybrids involving *A. elongatum* show a higher degree of ***Agropyron*** dominance than do hybrids involving *A. glaucum*. This is probably to be expected on the basis of chromosome number of the ***A. elongatum*** pollen ($n = 35$) as compared to ***A. glaucum*** pollen ($n = 21$), especially since considerable autosyndesis occurs among ***A. elongatum*** chromosomes (8). Of the ***Triticum*** parents, the variety C.A.N. 1835 appears to have been least able to overcome ***Agropyron*** dominance.

Considering the characters individually, the expressions are for the most part either dominant or partially dominant ***Agropyron***. There are, however,



1. *Unstimulated and stimulated ovaries of Crail Fife pollinated with A. junceum.* 2. *Hybrid seeds from Mindum pollinated with A. elongatum.* 3. *Root systems extracted from special containers. (Note yard stick). Left to right, A. elongatum (second year), Kharkov × A. elongatum (second year), Kharkov (fall sown, mature).*

TABLE VII

DESCRIPTIONS OF CERTAIN F_1 PLANTS SUMMARIZED IN TERMS OF RELATIVE DEGREES OF DOMINANCE, TRANSGRESSIVE EXPRESSION, OR HYBRID VIGOR

Character	*A. glaucum*					*A. elongatum*					Total
	Lutescens 0.62	C.A.N. 1835	Kharkov	*Mindum*	Vernal emmer	Lutescens 0.62	C.A.N. 1835	Kharkov	Mindum	Vernal *emmer*	
Life tenure	A	A	A	A	A	A	A	A	A	A	
Vegetative period	XA, H	XA	XA, H	XA	XA, H	XA, H	XA, H	XA, H	XA, H	XA, H	
Time of flowering	IA	IA	A	IA	IA	IA	IA	A	IA	IA	
Heading-to-flowering period	A	A	A	A	A	A	A	A	A	A	
Posture juvenile	IA	IA	I	IA	IA	IA	IA	I	IA	IA	
Posture mature	IA	A	A	A	IA	A	A	A	I	IA	
Height	H	–	I	H	H	H	H	H	–	H	
Tillering	H	A	A	X	H	H	A	H	A	H	
Leafiness	H	IA	IA	X	H	IA	IA	IA	H	IA	
Hollowness or solidity of stem	A	IA	IT	I	IA	IA	IA	IA	IA	I	
Reaction to ergot	XA	A	XA	A	XA	–	–	–	–	–	
Seminal rootlet number	IA	IA	IA	IA	IA	IA	IA	IA	IA	IA	
Mature root, extent	–	–	–	–	A	A	–	A	–	A	
Mature root, texture	–	–	–	–	I	I	–	I	–	I	
Coleoptile color	–	IA	IA	IA	IA	–	IA	IA	IA	IA	
Shattering of rachis	IA	IA	IA	IA	–	IA	IA	IA	IA	–	
Adherence of glumes to seed	–	–	–	–	–	IA	IA	IA	IA	IA	
Spike density	IT	I	I	I	I	I	IA	IA	IA	IA	
No. of spikelets	A	A	A	A	I	A	A	A	XA	XA	
No. of florets	A	A	A	A	A	IA	IA	IA	T	IA	
Awning	I	A	IA	IA	IA	A	A	IA	IA	A	
Secondary glume shoulder	A	A	IA	–	–	IA	–	I	–	XA	
Secondary glume beak length	T	A	I	A	T	I	A	I	I	I	
Secondary glume keel	T	X	T	T	T	A	A	I	A	I	
Leaf length	I	XA	–	–	A	IA	IA	–	–	A	
Leaf width	IA	IA	I	IA	I	IA	IA	IA	A	A	
Leaf pubescence dorsal	IT	IA	IT	IT	I	I	I	–	–	XT	
Leaf pubescence ventral	I	XT	IT	IT	I	I	I	IA	I	I	
Leaf pubescence marginal	IA	I	I	I	IA	I	I	X	I	I	

Concluded on page 434

TABLE VII—*Concluded*

DESCRIPTIONS OF CERTAIN F_1 PLANTS SUMMARIZED IN TERMS OF RELATIVE DEGREES OF DOMINANCE, TRANSGRESSIVE EXPRESSION, OR HYBRID VIGOR—*Concluded*

Character	*A. glaucum*					*A. elongatum*					Total
	Lutescens 0.62	C.A.N. 1835	Kharkov	Mindum	Vernal emmer	Lutescens 0.62	C.A.N. 1835	Kharkov	Mindum	Vernal emmer	
Leaf venation number	IA	IA	I	IA	IA	I	IT	I	I	I	
Leaf venation barbing dorsal	I	IA	–	I	IT	A	X	–	–	A	
Leaf venation barbing ventral	–	–	–	–	T	–	–	A	–	I	
Leaf marginal barbing	A	XT	IT	I	IA	A	A	XA	IA	IA	
Leaf rigidity	I	IT	–	I	I	I	I	I	IA	I	
Auricle color	–	–	T	–	–	–	–	T	–	–	
Ligule length	IA	IA	A	I	I	A	A	IA	A	A	
Node number	H	–	I	I	I	A	A	A	IA	T	
Node pubescence	A	A	IA	–	A	A	A	IA	–	A	
Culm diameter	I	IA	I	IA	I	A	A	A	XA	XA	
Neck diameter	I	IA	I	–	I	A	A	A	X	X	
Frequencies of the various expressions:											
Dominance: *Agropyron* (A)	8	11	8	7	6	14	14	10	6	9	93
Intermediate—*Agropyron* (IA)	9	14	7	9	10	11	12	13	12	10	107
Intermediate (I)	7	2	10	8	11	8	4	7	5	9	67
Intermediate—*Triticum* (IT)	2	1	4	2	1	0	1	0	0	0	15
Triticum (T)	2	0	2	1	3	0	0	1	1	1	11
Total under dominance	28	28	31	27	31	33	31	31	24	29	293
Transgressive: indeterminate (X)	0	1	0	2	0	0	1	1	1	1	7
Agropyron (XA)	2	2	2	1	2	1	1	2	3	4	20
Triticum (XT)	0	2	0	0	0	0	0	0	0	1	3
Hybrid vigor (H)	5	0	1	1	4	3	2	3	2	3	24

a number of cases where the expression is for the most part intermediate, for example, spike density, leaf marginal pubescence, leaf rigidity, node number, culm and neck diameter in *A. glaucum* crosses; and beak length, leaf pubescence dorsal, ventral and marginal, venation number and leaf rigidity in *A. elongatum* crosses. There are only two characters for which *Triticum* can be said to express dominance, namely, secondary glume keel in *A. glaucum* crosses and auricle color in crosses of both types. (Auricle color was white in all parental materials except Kharkov, in which the auricle was edged with purple. The purple color was inherited as a dominant).

Transgressive expressions sometimes typify a character, for example, prolongation of vegetative period (probably related to hybrid vigor) in both *A. glaucum* and *A. elongatum* crosses, reaction to ergot in *A. glaucum* crosses, and culm and neck diameters in *A. elongatum* crosses involving tetraploid wheats.

Hybrid vigor is mainly confined to the quantitative characters, height, leafiness and tillering, and to prolongation of the vegetative period (which is considered to be essentially a condition of increased vigor).

Fertility in F_1 Hybrids

During the winter of 1935–1936, 291 F_1 hybrids were grown to maturity in the greenhouse and the spikes examined for seed. Percentage seed-set was expressed on the basis of the number of fully formed florets. The results are summarized in Table VIII.

TABLE VIII

SEED-SET DATA FROM F_1 PLANTS GROWN IN THE GREENHOUSE 1935–1936

Cross	No. of plants	No. of spikes	No. of florets	No. of seeds	Per cent seed-set	Per cent fertile plants	Range of fertility
Kharkov × *A. glaucum*	36	67	1,805	0	0	0	—
Lutescens 0.62 × *A. glaucum*	21	58	1,497	0	0	0	—
C.A.N. 1835 × *A. glaucum*	5	14	368	0	0	0	—
Mindum × *A. glaucum*	53	130	3,299	0	0	0	—
Vernal emmer × *A. glaucum*	53	241	6,537	0	0	0	—
Combined crosses with *A. glaucum*	168	510	13,506	0	0	0	—
Kharkov × *A. elongatum*	38	85	1,762	0	0	0	—
Lutescens 0.62 × *A. elongatum*	16	37	869	0	0	0	—
C.A.N. 1835 × *A. elongatum*	13	33	661	24	3.6	7.7	0 – 27.3
Mindum × *A. elongatum*	5	6	94	0	0	0	—
Vernal emmer × *A. elongatum*	51	30	386	0	0	0	—
Combined crosses with *A. elongatum*	123	191	3,772	24	0.6	0.8	0 – 27.3

The data in Table VIII show that no selfed seeds were obtained from F_1 plants, 168 in number, of crosses involving *A. glaucum*. Another dehiscence was not observed in any of these plants. One seed was obtained from a hybrid of Kharkov × *A. glaucum*; but since no dehiscence occurred on the plant and there was opportunity for accidental backcrossing, it is believed

that the seed originated through backcrossing. The plant grown from the seed in question failed to head the following summer and died during the following winter.

In crosses involving *A. elongatum*, one plant (S4) among C.A.N. 1835 hybrids shed pollen freely and produced 24 seeds, giving a percentage of 27.3. All other plants showed complete non-dehiscence of anthers and no further seed was set.

After the hybrids growing in the greenhouse had been examined for seed they were cut back, cloned, and transplanted to the field in the spring of 1936. Under field conditions the plants grew more vigorously, tillering well and producing large spikes in great abundance. (An exception was the cross Mindum × *A. glaucum*, which grew very poorly in the field.) In crosses involving *A. elongatum* and C.A.N. 1835, Lutescens 0.62, and Kharkov, anther dehiscence in varying degrees was observed to be fairly general. As in the greenhouse, anther dehiscence was not observed among hybrids involving *A. glaucum*. The seed-set data are summarized in Table IX. Percentage seed-set is expressed on the basis of number of spikelets.

TABLE IX

SEED-SET DATA FROM F_1 PLANTS GROWN IN THE FIELD IN 1936

Cross	No. of plants	No. of spikes	No. of spikelets	No. of seeds	Per cent seed-set	Per cent fertile plants	Range of fertility
Kharkov × *A. glaucum*	23	1,768	29,324	0	0	0	—
Lutescens 0.62 × *A. glaucum*	25	2,694	39,334	0	0	0	—
C.A.N. 1835 × *A. glaucum*	4	281	4,342	0	0	0	—
Mindum × *A. glaucum*	3	262	4,192	0	0	0	—
Vernal emmer × *A. glaucum*	88	6,547	101,474	0	0	0	—
Combined crosses with *A. glaucum*	143	11,552	178,666	0	0	0	—
Kharkov × *A. elongatum*	36	3,560	49,973	146	0.3	30.6	0– 20.7
Lutescens 0.62 × *A. elongatum*	24	2,670	36,253	4,260	11.8	75.0	0–118.4*
C.A.N. 1835 × *A. elongatum*	13	3,130	47,054	3,549	7.5	84.6	0– 62.7
Mindum × *A. elongatum*	3	240	4,282	0	0	0	—
Vernal emmer × *A. elongatum*	37	2,225	28,800	0	0	0	—
Combined crosses with *A. elongatum*	113	11,825	166,362	7,955	4.8	35.4	0–118.4

* *The occurrence of percentages of fertility of over 100 is due to the fact that fertility percentages are calculated on the basis of number of spikelets rather than number of florets.*

A very considerable increase in fertility of field material over greenhouse material is demonstrated by the data in Table IX. The crosses, Kharkov × *A. elongatum*, Lutescens 0.62 × *A. elongatum*, and C.A.N. 1835 × *A. elongatum* show marked increases as follows: in seed-set, from 0 to 0.3%, 0 to 11.8%, and 3.6 to 7.5%, respectively; in fertile plants, from 0 to 30.6%, 0. to 75.0%, and 7.7 to 84.6%, respectively; and in upper limit of range of fertility, from 0 to 20.7%, 0 to 118.4%, and 27.3 to 62.7%. (It should be noted that in the case of C.A.N. 1835 × *A. elongatum*, percentage seed-set was based on number of florets in calculating greenhouse data and on number of spikelets in calculating field data.)

The hybrid plant from C.A.N. 1835 × *A. elongatum*, designated as S4, which produced seed in the greenhouse, had been cloned into six individual plants, which under field conditions produced a total of 273 spikes, 4,256 spikelets and 2,670 seeds (seed-set, 62.7%). This was the second-highest percentage seed-set obtained, the highest being from a Lutescens 0.62 × *A. elongatum* hybrid, designated S22, which produced, from a clone of four plants, a total of 129 heads, 1,548 spikelets and 1,833 seeds (seed-set, 118.4%).

The great increase of anther dehiscence observed under field conditions as compared to greenhouse conditions is believed to be due to *(a)* the advantageous effect upon pollen-grain development of the more nearly optimum conditions of nutrition, illumination, etc., obtained in the field and to *(b)* the effect upon anther-wall breakage of such conditions as wind movement, alternations in humidity and temperature, etc., which are more pronounced in the field than in the greenhouse.

In 1937, further data on fertility were obtained in the field upon material identical with, comparable to, or in addition to that previously grown. The fertility data compiled on crosses involving *A. elongatum* are considered to be reasonably comparable to those collected in previous years, since the material was, in many cases, identical and in other cases directly comparable. The results are given in Table X.

TABLE X

SEED-SET DATA FROM F_1 PLANTS GROWN IN THE FIELD IN 1937

Cross	No. of plants	No. of spikes	No. of spikelets	No. of seeds	Per cent seed-set	Per cent fertile plants	Range of fertility
Dawson's G.C. × *A. glaucum*	85	2,742	44,205	0	0	0	—
Minturki × *A. glaucum*	3	35	490	0	0	0	—
Minhardi × *A. glaucum*	13	179	2,957	0	0	0	—
C.D. 1435 × *A. glaucum*	22	434	5,696	0	0	0	—
White Odessa × *A. glaucum*	89	2,387	44,036	0	0	0	—
Kharkov × *A. glaucum*	16	313	5,556	0	0	0	—
Lutescens 0.62 × *A. glaucum*	7	104	1,490	0	0	0	—
Black Persian × *A. glaucum*	12	202	3,588	0	0	0	—
Mindum × *A. glaucum*	32	398	5,454	0	0	0	—
Vernal emmer × *A. glaucum*	45	1,273	17,393	0	0	0	—
T. polonicum × *A. glaucum*	2	76	852	0	0	0	—
Combined crosses with *A. glaucum*	326	8,143	131,717	0	0	0	—
Kharkov × *A. elongatum*	25	5,239	88,636	796	0.9	60.0	0 – 77.8
Lutescens 0.62 × *A. elongatum*	21	996	12,217	2,702	22.1	57.1	0 – 134.7
C.A.N. 1835 × *A. elongatum*	8	201	2,424	993	41.0	37.5	0 – 124.8
Mindum × *A. elongatum*	5	311	5,200	210	4.0	80.0	0 – 49.6
Vernal emmer × *A. elongatum*	109	7,370	94,125	400	0.4	33.0	0 – 11.2
Combined crosses with *A. elongatum*	168	14,117	202,602	5,101	2.5	41.7	0 – 134.7

The most striking feature of the 1937 results is the seed-sets obtained from the *A. elongatum* crosses involving the tetraploid wheats, Mindum and Vernal emmer, which previously had been completely sterile. Most of this material had grown to maturity the previous years without setting seed. A possible

explanation is that the plants did not become properly established early enough in the first year to permit coincidence between a certain developmental stage of the plant and a critical photoperiod to have a determinative influence on seed-set. Accidental backcrossing is not believed to be a tenable explanation.

In the *A. elongatum* crosses involving Lutescens 0.62 and C.A.N. 1835, made up of new clonal material from plants used in previous years, there was a marked increase in percentage seed-set and, paradoxically, a very considerable decrease in percentage of fertile plants. The two hybrid plants, S4 and S22, which were outstanding for fertility in 1936, retained their positions. In 1937, S4 gave 124.8 and S22 gave 134.7% seed-set, both figures representing increases over the previous year.

As previously, there was no definite indication of self seed-set in *A. glaucum* crosses, though a few seeds, thought to be backcrosses, were collected.

Cytological observations reported by Peto (8) show that bivalent and multivalent chromosomal associations are much more numerous in hybrids involving *A. elongatum* than in hybrids in which *A. glaucum* is concerned. This cytological difference is considered to be related to the characteristic fertility and sterility of the two groups of hybrids.

Relation of Pollen Viability to Anther Dehiscence and Fertility

Microscopic observations were made on the pollen of a wide range of F_1 plants in order to determine the relative proportions of functional and non-functional pollen grains produced by each plant. Since seed-set was known to be lower in the greenhouse than in the field for a given plant, pollen from cloned material grown both in the greenhouse and in the field was studied in several instances. These data were then studied in relation to anther-dehiscence and fertility data compiled from the same plants.

Anthers were collected a day or two prior to maturity, killed and fixed in Navashin's agent, washed, run up to 70% ethyl alcohol for storage, and stained as temporary preparations in Belling's iron-aceto-carmine. Five counts were made on five different anthers from each plant.

Data on type of pollen, anther dehiscence, and fertility from five selected plants are summarized in Table XI. The term "good" was applied to pollen grains normal in size and in nuclear and cytoplasmic contents. All other grains were considered as "bad".

It may be concluded from Table XI that: *(a)* there is a far greater proportion of bad pollen in plants grown in the greenhouse than in clones of the same plants grown in the field. *(b)* There is a correlation between dehiscence and the ratio of good to bad pollen. Ratios of 1 : 2.4 and lower proportions of bad give dehiscence, while ratios of 1 : 4.1 and higher proportions of bad give non-dehiscence. *(c)* There is a direct correlation between proportions of good pollen and seed-set when the proportions are sufficient to give anther dehiscence.

TABLE XI

DATA ON THE PROPORTIONS OF GOOD AND BAD POLLEN IN COMPARISON WITH ANTHER DEHISCENCE AND PERCENTAGE SEED-SET

Material (F_1)	Where grown	Type of pollen	Pollen counts					Total	Ratio good : bad	Condition of anther	Per cent seed-set
			1	2	3	4	5				
C.A.N. 1835 × *A. elongatum* (S4)	Greenhouse	Good	154	130	137	265	330	1016			
		Bad	500	500	500	465	500	2465	1 : 2.4	Dehiscent	27.3
C.A.N. 1835 × *A. elongatum* (S4)	Field	Good	575	770	518	647	638	3148			
		Bad	150	200	150	200	200	900	1 : 0.3	Dehiscent	108.9
Lutescens 0.62 × *A. elongatum* (S22)	Greenhouse	Good	0	0	0	0	0	0			
		Bad	500	500	500	500	500	2500	1 : ∞	Non-dehiscent	0
Lutescens 0.62 × *A. elongatum* (S22)	Field	Good	637	511	122	592	430	2292			
		Bad	398	252	98	341	500	1589	1 : 0.7	Dehiscent	118.4
Lutescens 0.62 × *A. elongatum* (S23)	Greenhouse	Good	79	53	243	95	98	568			
		Bad	500	203	500	500	500	2303	1 : 4.1	Non-dehiscent	0
Lutescens 0.62 × *A. elongatum* (S23)	Field	Good	294	787	221	1010	819	3131			
		Bad	160	500	82	500	300	1542	1 : 0.5	Dehiscent	63.6
Dawson's G.C. × *A. glaucum* (32)	Greenhouse	Good	28	14	15	23	17	97			
		Bad	500	276	500	500	500	2276	1 : 23.5	Non-dehiscent	0
Dawson's G.C. × *A. glaucum* (32)	Field	Good	34	33	49	37	38	191			
		Bad	500	430	500	500	425	2355	1 : 12.3	Non-dehiscent	0
Lutescens 0.62 × *A. elongatum* (S17)	Greenhouse	Good	388	118	194	9	144	823			
		Bad	412	500	424	500	500	2336	1 : 28.4	Non-dehiscent	0
Lutescens 0.62 × *A. elongatum* (S17)	Field	Good	435	416	0	510	292	1653			
		Bad	539	500	500	500	500	2539	1 : 1.5	Dehiscent	2.2

It is believed that internal pressure caused by expansion of the mass of developing pollen grains is an important factor in anther dehiscence. When there is a relatively large proportion of large rigid cells (good pollen) sufficient pressure is exerted to give dehiscence; but when there is a smaller proportion of these cells the pressure is less, and even further diminished by the collapse of the less rigid cells (bad pollen), now in greater proportion. The combined effect is the reduction of internal pressure below the point where it is an effective factor in anther dehiscence.

On the basis of the above data, together with cytological data from Table I of Peto's report (8), tentative statements may be made on the mechanism involved in the fertility-sterility relation. In crosses involving *A. elongatum*, which are more or less fertile, the proportions of bivalent and multivalent chromosomal associations are much higher than in crosses involving *A. glaucum*, which are sterile. The degree of chromosomal association is directly related to the degree of efficiency with which chromosomes are distributed to daughter cells in meiotic divisions, which in turn is directly related to the proportions of good and bad pollen produced, and these in turn are, as indicated above, related to anther dehiscence. In *A. glaucum* crosses, the degree of chromosomal association is below the minimum value required to set into effective operation the series of events leading to anther dehiscence. *A. elongatum* crosses are fertile in proportion to the degree of efficiency given this series of events by the different degrees of chromosomal association.

General Discussion with Particular Reference to Russian Investigations

The primary objective of the *Triticum-Agropyron* hybridization project is the production of new and superior types of forage plants adapted to the drier areas of western Canada. Such plants should be large-seeded to facilitate seeding and harvesting; they should have extensive fibrous root systems with good soil binding properties; they should be perennial and resistant to drought and cold; and they should give a good yield of palatable, nutritious forage. The parents used in these crosses embrace, collectively, all of these qualities. It remains to be seen, however, how near it will be possible to combine ideally these characteristics through selection in the segregating generations. Theoretically, since the perennial habit and clonability of the F_1 plants permit unlimited F_2 populations, only sterility and close genetic linkages stand in the way of the ultimate attainment of this objective.

Besides the primary objective, a number of other breeding possibilities are being kept in mind for future work, mainly with the co-operation of cereal workers. It will suffice here to enumerate these as follows: (i) the production of a perennial wheat that may be bred for feed-grain purposes and perhaps eventually for milling purposes; (ii) the production of a biennial wheat that, either directly or through further crossing, may provide a winter wheat of hardiness superior to existing varieties; and (iii) the isolation of segregate types that may be used as breeding materials for general cereal improvement along the lines of disease resistance, drought resistance, strength of straw, etc.

The original investigation on *Triticum-Agropyron* hybrids began at the Central Station of Plant Breeding and Genetics, Saratov, Russia, in 1930, when N. V. Tzitzin obtained the first hybrids (17). With the creation of perennial wheats as the objective, the work was soon being carried on at several stations in different agricultural regions of the U.S.S.R. To date a score or more of different workers have published results on various phases of the general program of perennial wheat breeding. Without attempting a complete summarization, this older and more extensive work will be considered briefly in comparison with our results and in relation to the prospects of attaining our objectives.

The Russian workers have been successful in crossing five *Agropyron* species with *Triticum*, namely, *A. glaucum*, *A. elongatum* (two forms, with chromosome complements of $2n = 56$ and $2n = 70$, respectively), *A. trichophorum*, *A. junceum* and *A. repens* (11). In early reports *A. intermedium* was also mentioned, but this form was later found to be identical with *A. glaucum* (14). We have been unable to obtain crosses with *A. repens*, although a total of 1,442 *Triticum* florets have been cross-pollinated. We have not tried *A. trichophorum*.

The Russian descriptions of F_1 plants resemble those of the present paper, although greater emphasis is given to *Agropyron* dominance by some authors (13, 17). The great variation among F_1 plants of the same cross is attributed to heterozygosity of the *Agropyron* parents (2, 14), which has led to the use of individual *Agropyron* plants in hybridization (11). The Russian observations on relative fertility among F_1 plants of various crosses agree essentially with the present report (2, 14, 15).

Studies in the U.S.S.R. upon advanced generations indicate that there are excellent possibilities of obtaining constant, perennial forms of superior drought and winter resistance that are earlier than annual wheat and that give high yields of good quality grain. The possibility of obtaining reasonably true-breeding, perennial lines possessing important *Triticum* characteristics was in 1934 demonstrated in an advanced, selfed generation of *T. vulgare* × *A. elongatum* (15). The problem then became one of isolating better-quality types among such lines.

Tzitzin (10) reported the isolation in 1934–1935 of constant, good-yielding, perennial lines with wheat-like seeds. In their second year of growth these lines ripened much earlier than annual wheats, indicating possibilities as a crop for agricultural zones of short growing season. Many of the hybrids inherited the extensive *Agropyron*-type root and proved to be more drought resistant and winter hardy than standard wheats. There were indications that hybrids involving *A. elongatum* had inherited the alkali-tolerance characteristic of this species.

Selecting presumably from backcross generations, Veruschkine (16) obtained several lines of constant, annual wheat types which were cytologically stable ($2n = 42$), markedly disease resistant and characterized by unusually high protein content of the grain (up to 21%). The bread baked from bulked

hybrid grain was considered to be superior to that baked from the standard wheat, Lutescens 0.62, while bread from certain individual hybrids was much higher in quality.

Veruschkine (16) also described intermediate types that, although dying in the field at Saratov, have the capacity to renew growth, indicating that they might behave as perennials in a milder climate. These forms represent a new octoploid wheat ($2n = 56$). They are disease resistant and, though smaller seeded, give higher yields than standard wheats. The protein content ranged from 22.3 to 24.5% in different lines, as compared with 17.2% for Lutescens 0.62, the standard wheat.

In accordance with Meister's belief, Veruschkine (16) considers that the origin of new types among these hybrids is not merely a result of genetic recombination, but also of mutation induced by the remote nature of the crosses.

Biochemical studies by Samsonov (9) and others have shown that the *Agropyron* parents have a much higher gluten content than standard wheats, and that *Agropyron* flour produced a loaf of normal wheat quality. The flour from high-yielding, annual, F_5 hybrids of Lutescens 0.62 × *A. glaucum* proved to be of excellent baking quality. These tests were considered to indicate that hybrids might be obtained that would replace standard varieties for milling, and it was suggested that biochemical examination of hybrid lines should precede selection work.

Udoljskaja (12) established the existence of two biological types of drought resistance in wheat. In plants of Type I, under deficient water supply, there is a loss of turgor and retarded development in the form of deferred shoots. Upon resuming adequate watering the deferred shoots revive, the plants recover rapidly, develop at an increased rate and mature with the controls. This type is very resistant at the tillering stage but very susceptible at the heading stage. Plants of Type II, under deficient water supply, lose their leaves, maintain turgor in the main stem, and do not show retarded development. This type is fairly resistant at the tillering stage and very resistant at later stages.

Studying hybrid plants growing in culture and in the field, Udoljskaja found that *Agropyron* dominance in the F_1 hybrids masked the type of resistance inherited from wheat. These types of resistance were expressed in later generations, however, and among the F_6 hybrids a number of lines were found to be superior to wheat in drought resistance. Hybrids involving winter wheats possessed Type I resistance.

Blinkova (3) studied winter hardiness in parental *Agropyron* species and concluded that they possessed exceptional winter hardiness owing to several physiological and biochemical properties that are transmitted in varying degrees to the *Triticum-Agropyron* hybrids. Analysis of the hybrids shows that *(a)* they are characterized by resistance of plasmic proteins to low temperature and by mobility of the albumen complex; *(b)* they are more

tolerant of conditions of hardening than the most hardy wheat and rye; and *(c)* they expend reserve nutrient with greater economy and secrete carbohydrate more vigorously during hardening than do hardy wheat and rye. Hybrids that failed to head during the first year after sowing were found to have high plasmic resistance to low temperature. It was concluded that there is a correlation between developmental stages and changes in plasmic proteins.

Although the Russian investigations have not been conducted from the forage point of view it is inevitable that the results should have important bearings upon a forage-breeding project based on the same crosses. The frequent isolation by Russian workers of constant, fertile, resistant and highly-productive perennial forms is extremely important to the forage project because they are qualities toward which forage selection must be directed. The Russian work on drought and winter resistance applies directly to forage breeding. The high protein content of the grain of Russian selections indicates possibilities of developing a forage plant which might yield also a valuable grain-concentrate for feeding purposes. There remains unanswered, however, the very important question of the possibility of isolating suitable forage types. It can only be said that there is nothing in the Russian literature nor in the present report that should discourage a full attack upon the forage-breeding problem.

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CYTOLOGY OF POPLAR SPECIES AND NATURAL HYBRIDS[1]

BY F. H. PETO[2]

Abstract

Complete analyses of pollen-mother-cell nuclei at first metaphase, percentage good pollen, pollen diameter and pollen-size distribution were determined for the following poplar species and natural hybrids: *Populus grandidentata* Michx., *P. tremuloides* Michx., *P. eugenei* Simon Louis, *P. alba* L., *P. canescens* Sm., natural hybrids of *P. alba* × *P. grandidentata* and of *P. alba* × *P. tremuloides*.

Both of the *P. alba* and two of the four *P. canescens* trees examined were triploids ($2n = 57$) while all other trees examined were diploids ($2n = 38$). Meiotic observations on the natural hybrids indicated a high degree of homology between the chromosomes of *P. alba* and the native aspens (*P. grandidentata* and *P. tremuloides*), since 17 to 19 bivalents were usually found at first metaphase. In collections from one triploid *P. canescens* and two diploid *alba-grandidentata* hybrid trees, failure of a high proportion of the chromosomes to pair was attributed to genetic factors limiting pairing, rather than to non-homology.

Pollen characters such as percentage good pollen, pollen diameter, and pollen size distribution were, in most cases, not indicative of the chromosome number or pairing relations at first metaphase. Consequently, triploids could not be detected by pollen observations under the conditions of this experiment. In spite of the lack of correlation between first metaphase and pollen observations, abnormally large pollen grains were observed in collections from several of the trees, and these were considered to contain the diploid or unreduced chromosome complement. The tendency for the poplars to produce unreduced pollen grains probably accounts for the number of triploid trees discovered in Canada and Sweden.

Introduction

Natural hybrids between European *Populus alba* and the two native aspens, *P. grandidentata* and *P. tremuloides*, are found in a number of localities in the vicinity of Ottawa (Table I). These trees were discovered by Dr. C. Heimburger of the Dominion Forest Service, Ottawa, subsequent to his early work on the artificial hybridization of these species. A preliminary report on the hybridization studies was published in 1936 (5), and a detailed description of the natural hybrids and their economic possibilities will be published in the near future. The rapid growth and excellent quality of certain of the *alba-grandidentata* hybrids have indicated the possibilities of producing, by artificial hybridization, rapid-growing and disease-resistant trees for match stock, pulpwood, and shelter belts. Additional favorable qualities may also be induced by the production of triploid and tetraploid varieties. Promising leads in this regard were given by Nilsson-Ehle (9), Blomqvist (2), and Melander (6) by their recent discovery in Sweden of three strains of a giant

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada.
[2] *Cytogeneticist, National Research Laboratories, Ottawa.*

triploid form of *P. tremula* that exhibit very rapid growth rates and in one case (6) resistance to heart rot.

The present study was undertaken to provide cytological information on certain natural hybrids and parental species that might be of value as a guide in the breeding program. Determination of the chromosome number and a complete analysis of meiotic associations were made on the pollen mother cells of 23 trees. The male catkins were fixed at the proper stage in absolute alcohol 3 parts and glacial acetic acid 1 part, stored in 70% alcohol, smeared, and stained in aceto-carmine.

TABLE I

LOCATION OF POPLAR SPECIES AND NATURAL HYBRIDS STUDIED

Species	Tree No.	Native or introduced (N or I)	Place of collection
P. grandidentata	G 1	N	Petawawa, Ont.
P. tremuloides	T 1	N	Edmonton, Alberta.
P. tremuloides	T 2	N	Calgary, Alberta.
P. eugenei	—	I	Arboretum, Central Experimental Farm, Ottawa.
P. alba	A 1	I	Gatineau Mills (near Hull, Que.)
P. alba var. *nivea aureo-intertexta*	A 2	I	Arboretum, Central Experimental Farm, Ottawa.
P. canescens	C 3	I	Ottawa (Eastview), Ont.
P. canescens	C 4	I	Hull, Aylmer Road, Que.
P. canescens	C 5	I	Farmers Rapids, Gatineau River, Que.
P. canescens	C 7	I	Ottawa, Ont.
P. canescens	C10	I	Arboretum, Central Experimental Farm, Ottawa.
P. alba × *P. grandidentata*	AG 7	N	Ottawa (Rideau Canal), Ont.
P. alba × *P. grandidentata*	AG 8	N	Petawawa, Ont.
P. alba × *P. grandidentata*	AG10	N	Bank and Clemow Sts., Ottawa, Ont.
P. alba × *P. grandidentata*	AG31	N	Station A, Longueuil, Que.
P. alba × *P. grandidentata*	AG34	N	Hull, Aylmer Road, Que.
P. alba × *P. grandidentata* (monoecious)	AG35	N	Hull, Aylmer Road, Que.
P. alba × *P. grandidentata*	AG36	N	Hull, Aylmer Road, Que.
P. alba × *P. grandidentata*	AG37	N	Hull, Aylmer Road, Que.
P. alba × *P. grandidentata*	AG39	N	Hull, Aylmer Road, Que.
P. alba × *P. grandidentata*	AG42	N	Hull, Aylmer Road, Que.
P. alba × *P. grandidentata*	AG44	N	Hull, Aylmer Road, Que.
P. alba × *P. grandidentata*	AG45	N	Hull, Aylmer Road, Que.
P. alba × *P. grandidentata*	AG46	N	Hull, Aylmer Road, Que.
P. alba × *P. tremuloides* (monoecious)	AT10	N	Bank and Isabella Sts., Ottawa.
P. alba × *P. tremuloides*	AT11	N	Ottawa, Ont.
P. alba × *P. tremuloides*	AT12	N	Hull, Aylmer Road, Que.

The branches of the male trees were cut in midwinter and stored in a snow bank until needed, or wrapped in moist paper and stored in a refrigeration chamber at about 36° F. On removal from cold storage, the branches were placed in beakers containing a small quantity of water and kept in the greenhouse at about 65° F. Those collected in 1937 were kept in the laboratory at 70 to 75° F. The proper stage for examination was reached from 50 to 90 hr. after removal from cold storage. Since all the anthers on the catkin

pass through the metaphase stage in a few hours, frequent examinations had to be made to procure material at the proper stage.

The diameter and size distribution of the mature pollen were also determined for most of these trees. The object was to determine whether there was a sufficiently close correlation between chromosome number and pollen size to permit use of the latter to detect triploids and higher polyploids. It was also hoped that pollen examinations would give some indication of meiotic behavior and reveal the frequency of occurrence of unreduced pollen grains. The anthers were collected just prior to pollen shedding or in some cases the pollen was dusted directly into vials. The same method of fixation and staining as used for the pollen-mother-cell study was employed. The pollen was gently teased out of the anthers without undue crushing. Pollen distortion through cover-slip pressure on the pollen grains was avoided by supporting the edges of the cover slip by very narrow strips of thin cover-slip glass.

Cytological and Pollen Observations

Native Aspens

P. grandidentata and *P. tremuloides* regularly formed 19 bivalents at first metaphase as shown in Table II and illustrated in Figs. 1 and 2. The chromosome number of *P. tremuloides* had previously been determined by Erlanson and Hermann (4), who also observed 19 bivalents at first metaphase. However, these authors observed a heteromorphic bivalent that they conclude

TABLE II

MEIOTIC ASSOCIATIONS IN POPLAR SPECIES AND NATURAL HYBRIDS

Species	Tree No.	(2n) Chr. No.	Nuclei examined	Univalents	Bivalents	Trivalents
P. grandidentata	G 1	38	8	0	19	0
P. tremuloides	T 1	38	5	0	19	0
P. tremuloides	T 2	38	5	0	19	0
P. eugenei	—	38	4	4.0	17	0
P. alba	A 1	57	5	12.0	13.8	5.8
P. alba var. *n.a.i.*	A 2	57	4	5.75	5.75	13.25
P. canescens	C 3	38	10	1.0	18.5	0
P. canescens	C 5	57	10	51.6	2.7	0
P. canescens	C 7	57	5	15.2	12.2	5.8
P. alba × *P. grandidentata*	AG 8	38	10	0	19.0	0
P. alba × *P. grandidentata*	AG10	38	10	16.2	10.9	0
P. alba × *P. grandidentata*	AG31	38	10	0.4	18.8	0
P. alba × *P. grandidentata*	AG34	38	5	1.6	18.2	0
P. alba × *P. grandidentata*	AG35	38	10	1.8	18.1	0
P. alba × *P. grandidentata*	AG36	38	10	34.4	1.8	0
P. alba × *P. grandidentata*	AG37	38	5	0.4	18.8	0
P. alba × *P. grandidentata*	AB39	38	10	0.8	18.6	0
P. alba × *P. grandidentata*	AG42	38	10	3.7	17.0	0.1
P. alba × *P. grandidentata*	AG44	38	7	0.86	18.6	0
P. alba × *P. grandidentata*	AG45	38	10	1.8	18.1	0
P. alba × *P. grandidentata*	AG46	38	10	0.5	18.6	[illegible]
P. alba × *P. tremuloides*	AT10	38	10	1.8	18.1	0
P. alba × *P. tremuloides*	AT11	38	10	5.2	16.4	0
P. alba × *P. tremuloides*	AT12	38	10	0.4	18.8	0

represents the sex chromosomes. Blackburn and Harrison (1) and Meurman (7) also report the presence of a pair of heterochromosomes that they conclude are involved in sex determination. Although a special study of sex chromosomes has not been made, no definitely heteromorphic pair has been found consistently, in spite of the fact that the best preparations were sufficiently clear to permit complete analysis of side-view metaphase plates. In certain of the hybrids, however, heteromorphic bivalents were noticed, which could best be explained by the presence of structural differences evolved in species differentiation. A further careful study of the native aspens will be made in the near future in an attempt to settle definitely whether morphologically distinct sex chromosomes are present.

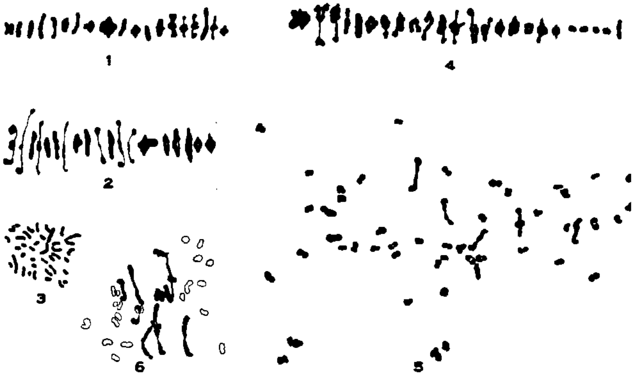

FIG. 1. *P. grandidentata (G1), 19 bivalents at metaphase, ×1700.* FIG. 2. *P. tremuloides (T1), 19 bivalents at metaphase, ×1700.* FIG. 3. *P. alba × P. grandidentata (AG10), root tip division, 38 chromosomes, ×2300.* FIG. 4. *P. alba var. nivea aureo-intertexta (A2), triploid, 5 univalents, 5 bivalents, 14 trivalents, ×1700.* FIG. 5. *P. canescens (C5), triploid, 47 univalents, 5 bivalents, ×1700.* FIG. 6. *P. alba × P. grandidentata (AG10), 29 univalents and 9 bivalents, ×1700.*

P. grandidentata and *P. tremuloides (G1, T1)* both produced a high percentage of apparently good pollen but exhibited a very wide range in pollen diameter (Table III) compared to the narrow range of variability for *T2*. The presence of pollen grains of 36 to 37 units in diameter, compared to the mean diameter of about 23 units, suggests that these grains may possess at least the diploid chromosome number. This range of distribution may be much greater than that occurring under natural conditions, as the laboratory temperatures of 70 to 75° F., to which these branches were exposed, were considerably higher than the outdoor temperatures at which the reduction division occurs. Reduction and separation of the chromosomes at anaphase appear

TABLE III

CHROMOSOME NUMBER, POLLEN DIAMETER, AND SIZE DISTRIBUTION OF GOOD POLLEN FROM POPLAR SPECIES AND NATURAL HYBRIDS

Species	Tree No.	Year	Chr. No.	Good pollen, %	Mean diam.	S.D. of single observations	Class values (1 unit = 1.1μ)													
							14–15	16–17	18–19	20–21	22–23	24–25	26–27	28–29	30–31	32–33	34–35	36–37	38–39	40–41
P. grandidentata	*G 1*	1937	38	97.5	23.9	3.00		1	6.5	6	36.5	19.5	22	3.5	1.5	0	0.5	0.5		
P. tremuloides	*T 1*	1937	38	100	23.6	2.39			5	6.5	45.5	30	12	0	.5	0	.5			
P. tremuloides	*T 2*	1938	38	87	28.4	1.69						4	23	37	20	3				
P. eugenei		1938	38	68	25.4	4.98		1	2	7	9	17	13	14	2	1	0	1	1	
P. alba	*A 1*	1938	57	83	28.2	2.21					4	5	18	28	25	3				
P. alba nivea aureo-intertexta	*A 2*	1938	57	94	26.7	2.14					5	16	41	24	7	1				
P. alba nivea aureo-intertexta	*A 2*	1937	57	98.5	27.3	2.38			.5	.5	9	12	40.5	18.5	15	2	.5			
P. canescens	*C 3*	1938	38	94	23.0	1.83				18	47	20	6	3						
P. canescens	*C 4*	1938		81	25.7	2.26				2	12	21	31	11	4					
P. canescens	*C 5*	1937	57	96	27.7	3.59			1	1	7.5	12	36	17.5	13	1.5	3.5	1	2	
P. alba × *P. grandidentata*	*AG 7*	1937	38	50.5	24.0	3.44	1	0	7.5	3	11	7	17	3	.5	.5				
P. alba × *P. grandidentata*	*AG 8*	1938	38	73	22.4	1.97			1	23	32	10	6	1						
P. alba × *P. grandidentata*	*AG35*	1938	38	93	21.2	1.86		3	10	42	31	4	3							
P. alba × *P. grandidentata*	*AG36*	1938	38	86	24.7	2.93				7	25	23	17	10	4					
P. alba × *P. grandidentata*	*AG39*	1938	38	92	27.8	3.45					4	16	31	20	10	5	3	1	1	1
P. alba × *P. grandidentata*	*AG42*	1938	38	52	26.7	4.62		1	0	2	10	11	7	6	6	4	2	3		
P. alba × *P. grandidentata*	*AG44*	1938	38	86	25.7	2.76				6	12	22	28	12	4	2				
P. alba × *P. grandidentata*	*AG46*	1938	38	57	24.8	2.70				4	17	13	16	5	2					
P. alba × *P. tremuloides*	*AT10*	1937	38	98	22.5	2.34	1.5	1.5	12.5	8.5	45	20.5	8.5							
P. alba × *P. tremuloides*	*AT10*	1938	38	90	23.0	2.15		1	5	6	47	22	7	2						
P. alba × *P. tremuloides*	*AT12*	1938	38	75	26.7	2.71			1	1	6	14	24	18	10	1				

200 pollen grains of each species were measured in 1937 and 100 of each in 1938.

to be normal in the material examined. It therefore appears likely that the large pollen grains were formed through failure of cytokinesis and microspore wall formation. The normal behavior during reduction division would appear to favor this type of abnormality. Cytokinesis and wall formation do not always occur in the interphase between the first and second divisions, as is the case in many plants, but are frequently delayed until after telophase of the second division. The delay or absence of normal wall formation may, under certain conditions, permit restitution nuclei to be formed. This, of course, would result in the large pollen grains observed. If this tendency to produce diploid pollen grains is general in nature or can be induced under certain conditions, an excellent opportunity exists to produce triploid or tetraploid plants. The natural occurrence of autotriploid plants of *P. tremula* in Sweden (2, 6, 9) and the discovery in Canada of triploid trees of *P. alba* and *P. canescens* indicate that the production of diploid pollen is not uncommon in nature.

The pollen diameter of *P. tremuloides* from Calgary was significantly larger than that of *P. tremuloides* from Edmonton (Table III). This may be a true varietal difference but further observations must be taken to establish this point.

P. eugenei

This species originated as a seedling in the nursery of Simon Louis Frères at Plantières, near Metz, France, in 1832. Cansdale (3) states that Henry, Schneider, and Houtzagers are all agreed that the parents were *P. regenerata* and *P. nigra* var. *italica*. The chromosome number of the specimen examined was $2n = 38$. It was possible to find only four nuclei that could be completely analyzed and in these the number of univalents varied from 0 to 10. It was apparent that meiosis in this tree was much more irregular than in the native aspens; these irregularities were reflected in the pollen characters, as there was only 68% good pollen and the standard deviation of pollen diameter was the largest of any of the trees examined. Some very large pollen grains were observed and it is likely that these contained at least the diploid chromosome complement. The hybrid constitution of this tree probably accounts for the irregularities observed.

P. alba

Two male trees of this species *(A1, A2)* were examined. These trees differed considerably from the female trees of this species growing in the Ottawa district. Both were found to be triploid, whereas the female parental trees of the *alba-grandidentata* hybrids examined to date must be diploid, since these hybrids all have the diploid chromosome number.

The mean frequency of univalents, bivalents and trivalents of *A1* and *A2* is given in Table II. The total of the bivalents and trivalents in triploid nuclei should equal the haploid chromosome number of 19. Actually the values were 19.6 for *A1* and exactly 19 for *A2*. The slight excess in *A1* was probably due to a slight error in interpreting the metaphase configurations.

While the maximum number of configurations was present in the nuclei of both trees, an average of 13.25 trivalents was observed in *A2* and only 5.8 trivalents in *A1*. These results indicate that *A2* is an autotriploid and that *A1* may be a hybrid. This is further indicated by the yellowish color of the *A1* bark which is not common on the trees of *P. alba* growing in this district.

A *camera-lucida* drawing of all the configurations at first metaphase in a nucleus of *P. alba* var. *nivea aureo-intertexta* is shown in Fig. 4. A wide variety of types of trivalents was found, including Y, U, spoon, and chain types. A photomicrograph of the same nucleus is shown in Plate I, Fig. 3. Only a few of the configurations are in sharp focus, but it is possible to see the trivalent constitution of several of them.

The percentage of apparently good pollen in the two varieties of *P. alba* was surprisingly high for triploids, being 83 and 94%. The range of distribution of pollen sizes was narrow, with no evidence of unreduced pollen grains being produced. It was expected that triploids might be detected by pollen examination, since triploids in other plants usually have a high proportion of poor pollen with a wide range in pollen size. Such is not the case in these trees, although it is scarcely possible that all the pollen that is normal in appearance is equally viable.

The observations by Müntzing (8) and Tometorp (10) on the pollen of the *P. tremula* triploids found in Sweden form an interesting comparison to our own observations on the pollen of triploid *P. alba*. Both Müntzing and Tometorp examined pollen of the Lillö gigas type. The latter observed a distribution of pollen diameter of from 25μ to 42μ, which is comparable to that observed for *P. alba*, which was approximately 20μ to 38μ. In neither case was the distribution definitely bimodal. However, Müntzing obtained a definite bimodal distribution of the "Lillö" pollen and, although he did not give absolute values, it is apparent that the range of distribution was much wider than that recorded by Tometorp. Environmental influences probably account for the differences observed, as unreduced pollen grains may have been produced with a much higher frequency under the environmental conditions to which Müntzing's sample was subjected.

P. canescens

This species is believed to have originated by natural hybridization between the two European species, *P. alba* and *P. tremula* (11). These hybrid trees are very similar in many respects to the natural hybrids between *P. alba* and *P. grandidentata* found in Canada, although the shape of the leaves is sufficiently different for them to be distinguished. Two of the *P. canescens* trees examined proved to be triploids (*C5, C7*) with 57 chromosomes, while two others were diploid (*C3, C10*). Root-tip rather than meiotic counts were made on *C10* since saplings only were available for study. The total number of bivalent and trivalent associations in the nuclei of tree *C7* was 18, which approaches the theoretical maximum of 19. The proportion of the various

associations was very similar to that observed for *P. alba (A1)*, which was also suspected of being a hybrid. The other triploid tree of *P. canescens (C5)* exhibited on the average only 2.7 bivalents per nucleus with a range of from 0 to 7. It hardly seems likely that lack of homology among the three sets involved accounts for this high degree of asynapsis, and it is concluded that there must be genetic factors limiting pairing. A drawing and photomicrograph of first metaphase nuclei of this tree are shown in Fig. 5 and Plate I, Fig. 4. Certain of the univalents in this nucleus showed signs of splitting equationally. Other observations indicated that the univalents usually all divide at the first division. The abnormal meiotic behavior is reflected in the variability in pollen diameter from about 18 to 38 units and in the high standard deviation of 3.59. The distribution appears to be bimodal and it is likely that unreduced or triploid pollen grains are produced. The percentage of good pollen was surprisingly high, being 96%, but it is highly unlikely that all of such a high proportion could receive a sufficiently well-balanced genic constitution to be viable.

P. alba × *P. grandidentata*

The chromosome number and meiotic behavior of 12 natural hybrids of this cross were determined. The 2*n*-chromosome number was 38 in all of these trees, and an average of between 17 and 19 bivalents was observed at first metaphase in ten of the trees. Chiasmata frequencies were not determined in this material, but the proportion of ring to rod bivalents in these ten hybrids appears to be similar to that found in *P. grandidentata*. It is therefore concluded that a high degree of homology exists between the *alba* and *grandidentata* chromosomes. A typical nucleus of *AG39* containing 19 bivalents is shown in Plate I, Fig. 1. The pairing behavior of *AG10* and *AG36* was very different from that of the others. In the former, 16.2 univalents and 10.9 bivalents were observed, while 34.4 univalents and only 1.8 bivalents were observed in the latter. A nucleus of *AG36* exhibiting complete asynapsis is shown in Plate I, Fig. 2, and a nucleus of *AG10* with 9 bivalents and 20 univalents is shown in Fig. 6. It does not seem likely that the asynapsis observed in these two plants is caused by a low degree of homology between the parental chromosome complements, neither is it likely that it was caused by an unfavorable environment, since catkins from trees in the immediate vicinity and handled in the same manner exhibited normal pairing. We therefore conclude that this is another example of the operation of genetic factors limiting pairing.

Only two trivalents were observed in all the *alba-grandidentata* nuclei examined. This indicates that structural changes have played a relatively unimportant role in the evolutionary differentiation of these two species.

Examination of the pollen data in Table III shows a wide variation in pollen diameter (21.2 to 27.8 units). Not only do the means differ considerably but the standard deviation of single observations varies from 1.86 to 4.62. In addition, the proportion of good pollen also varied from 50 to 97%.

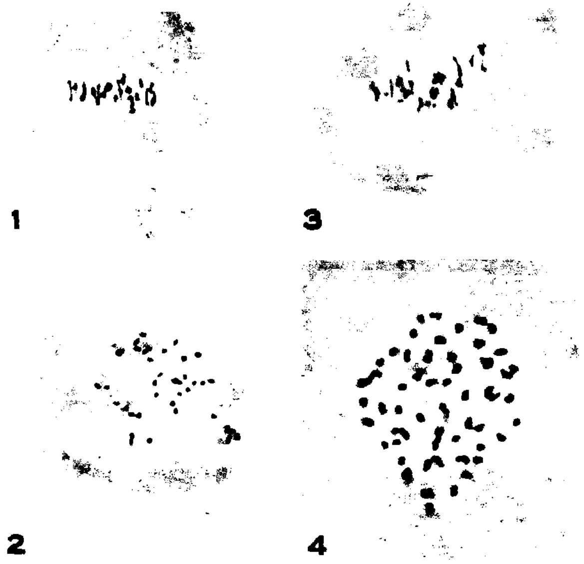

FIG. 1. *P. alba* × *P. grandidentata* (AG39), *metaphase I, 19 bivalents*, ×1040. FIG. 2. *P. alba* × *P. grandidentata* (AG36), *metaphase I, 38 univalents*, ×1040. FIG. 3. *P. alba var. nivea aureo-intertexta* (A2), *triploid, metaphase I, 5 univalents, 5 bivalents, 14 trivalents*, ×1040. FIG. 4. *P. canescens* (C5), *triploid, metaphase I, 53 univalents, 2 bivalents*, ×3280.

Prior to these observations, it was considered that any marked degree of asynapsis would probably be reflected in a definite reduction in the percentage good pollen, and an increase in the standard deviation of pollen diameter. This, however, was *not* the case in a plant such as *AG36*, which *(i)* exhibited almost complete asynapsis, *(ii)* produced 86% apparently normal pollen, *(iii)* showed a relatively low standard deviation of pollen diameter, and *(iv)* produced no very large pollen grains. Among the *AG* plants that exhibited normal meiotic pairing, wide variations were observed in the percentage good pollen, mean diameter and standard deviation. There was no definite correlation between these variables, except a tendency towards a positive correlation between mean diameter and standard deviation. This may be explained by the fact that the development of unreduced pollen grains would increase both the mean pollen size and the range of distribution. However, if the production of unreduced pollen grains was the only factor involved, the modal class for the haploid pollen grains should be similar for different *AG* trees, and some indications of a bimodal distribution would be found in those pollen samples with a wide size distribution. However, such was not the case, and other explanations must be sought. Inherent differences in growth vigor between trees might account for some of the differences in mean diameter and standard deviation. It should also be remembered that the catkins passed through their critical stages of gametophytic development under artificial conditions, since the branches were detached from the tree, stored, and then placed under warmer conditions than encountered in nature. These conditions might be expected to accentuate any inherent or developmental differences already present. The only clear-cut conclusion that can be arrived at from a study of these pollen data is that the trees *AG39* and *AG42* produce abnormally large pollen grains that probably contain the diploid chromosome complement.

AG35 was found to be a monoecious tree with both staminate and pistillate flowers in some of the catkins, the remainder being typically male. A very similar situation in *P. tremuloides* was described in detail by Erlanson and Hermann (4).

P. alba × *P. tremuloides*

These hybrids are not as common in this district as the *alba-grandidentata* hybrids. They appear to exhibit less hybrid vigor than the *AG* hybrids and do not appear to have as great economic possibilities. The meiotic behavior was determined for three trees. *AT10* and *AT12* gave very similar results, possessing 18.1 and 18.8 bivalents at first metaphase, while *AT11* had 16.4 bivalents.

Pollen data were procured in both 1937 and 1938 on *AT10*, and the results checked very closely for all characters. In this particular case the environmental differences seemed to have very little influence. The pollen of *AT12* differed from the other two collections in that the mean size was significantly higher, although the standard deviation was only slightly higher.

AT10 was observed to be monoecious with certain branches forming staminate catkins and others forming only pistillate catkins. It differed from the monoecious *alba-grandidentata* tree *AG35* in that perfect flowers were not observed.

Relation of Pollen Characters to Meiotic Behavior

One of the objects of this study was to determine the value of pollen studies as indicators of meiotic behavior and chromosome number. For example, it was expected that a marked degree of asynapsis would be reflected in a definite reduction in the percentage good pollen and a wider range of variation in pollen diameter. In addition it was expected that the pollen of triploids could be readily distinguished from the pollen of diploids on the basis of size. None of these expectations was realized. Diploid hybrids such as *AG36*, which exhibited almost complete asynapsis, appeared to produce a high percentage of good pollen, and the size distribution of this pollen was not abnormal. The expected meiotic irregularities in the triploids were observed, but the percentage of good pollen was high and in most cases the pollen size distribution as shown by the standard deviation values did not exceed many of the diploids that exhibited normal meiotic behavior. The final explanation of this situation must await further detailed studies on all stages of pollen development, but the observations to date appear to justify tentative conclusions.

Some of the univalents always divide equationally in the first division and wander at random in the second. An anaphase count was made at the first division in *AG36* ($2n = 38$) showing approximately 35 chromosomes at one pole and 22 at the other. This indicates that about 13 univalents split equationally and that irregular numerical segregation was occurring. Micro-nuclei were seen at the tetrad stage. Definite evidence of pollen degeneration was observed shortly after tetrad formation in *AG10*, and it seems likely that this degeneration may be very rapid. In the triploid varieties there was also evidence that some pollen degeneration occurred shortly after tetrad formation. Very little poor pollen was detected at pollen maturity, although a high percentage was expected. Careful examination of the pollen from these trees revealed a large number of tiny bodies which were too small to be recognized as degenerate pollen. Diploids with normal meiotic behavior did not possess nearly as many of these bodies. The tentative conclusion is that these tiny bodies probably are pollen grains in advanced stages of degeneration. Genetically unbalanced pollen grains probably degenerate very rapidly and to such an extent that it is impossible to determine their frequency at pollen maturity. Consequently only pollen grains that degenerated more slowly and could be recognized as pollen at maturity were included in counts of poor pollen.

Acknowledgment

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

III. CORRELATIONS BETWEEN NITROGEN AND SACCHARIFYING ACTIVITIES[1]

By J. Ansel Anderson[2], Henry R. Sallans[2], and C. Alan Ayre[2]

Abstract

Investigations made with samples representing 12 varieties of barley, grown at 12 experimental stations in Canada, show that fairly close intra-varietal correlations exist between the total nitrogen of barley and the saccharifying activities of the barley and of the malt made from it; and that these correlations are *closer than the corresponding correlations with nitrogen fractions. No inter-*varietal correlation exists between saccharifying activities on the one hand, and total nitrogen, alcohol-soluble nitrogen, or insoluble nitrogen, on the other, but inter-varietal correlations appear to exist between saccharifying activities and the more soluble nitrogen fractions.

The correlations between the nitrogen, or nitrogen fractions, and total barley saccharifying activity (papain method), and between the nitrogen, or nitrogen fractions, and free malt saccharifying activity (Lintner value), are closer than the corresponding correlations for free and latent barley saccharifying activities. Latent barley saccharifying activity is more closely correlated with total nitrogen than with any of the nitrogen fractions studied.

A study of the multiple correlations between malt saccharifying activity, and total nitrogen and 1000-kernel weight of barley, shows that the improvement resulting from the introduction of 1000-kernel weight as a second independent variable is very small.

Part I (1) of this series of papers described a study of total nitrogen and its distribution among protein fractions, made with 144 samples of barley representing 12 varieties grown at 12 widely separated experimental stations in Canada. In Part II (10) the results of determinations of the saccharifying activities of the same barley samples and of the malts made from them were reported. The relations between these two sets of data have now been examined and are described in the present paper.

The literature on barley contains a considerable amount of information on the relation between total nitrogen content and saccharifying activity. Bishop (5) found that, within each variety, a direct relation exists, and he developed equations for predicting the diastatic power of malts from data on the nitrogen content and 1000-kernel weight of the barleys from which the malts were made. He found, however, that this relation did not hold between varieties, *i.e.*, varieties that tend to be high in nitrogen content do not necessarily tend to produce malts of high diastatic activity. Anderson and Rowland (2) agree with these conclusions. Berglund (4) found some evidence of a relation but tends to be non-committal. Myrbäck (8), who determined the saccharifying activities of both barleys and malts, agrees that no relation exists between varieties and found only slight evidence of a relation within varieties.

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[2] *Biochemist, National Research Laboratories, Ottawa.*

A direct and rather more comprehensive investigation of this subject has recently been made by Chrzaszcz and Sawicki (6). They failed to find varietal differences in the amylase activities of barleys or malts and are thus unable to comment on the inter-varietal relation. Their general conclusions are stated as follows:— "Die Eiweissmenge in der Gerste ist auf deren wirkliche Amylasemenge ohne deutlichen Einfluss. Man kann höchtens feststellen, dass Gerstenproben mit grösserem Eiweissgehalt häufiger mehr Amylase als Gerstenproben mit geringem Eiweissgehalt besitzen."

No investigation of the relations between nitrogen fractions of barley and amylase activity has come to the authors' attention. The matter is of some importance owing to its bearing on hypotheses concerning the manner in which part of the amylase of barley is rendered unextractable by water. Thus Myrbäck and his co-workers (9 and papers cited therein), following the earlier suggestions of Ford and Guthrie (7) and Baker and Hulton (3), consider it probable that the "latent" amylase is bound to a high-molecular, insoluble protein compound, and that the amylase is set free by proteolytic hydrolysis. If this hypothesis is true, it seems possible that data substantiating it might be obtained by a study of the relations between nitrogen fractions and amylase activities.

Data and Methods

The experimental data were collected by analysis of 144 samples of barley and of the malts made from them. The samples represent 12 varieties grown at 12 widely separated experimental stations in Canada. A detailed description of the varieties and of the methods used in growing the samples was published in Part I of this series (1), which also contains the data on the total nitrogen, insoluble protein nitrogen, alcohol-soluble protein nitrogen, total salt-soluble nitrogen, salt-soluble protein nitrogen, and non-protein nitrogen of the barley samples. The results of determinations of free saccharifying activity, total activity (papain method), and latent activity (calculated by difference) of the barleys, together with data on the free saccharifying activity (Lintner value) of the malts, were published in Part II (10).

The relations between the six sets of nitrogen data and the four sets of amylase data were examined by means of correlation studies. In view of the space required, it seems inadvisable to present in detail the results of each of the 24 separate studies. We have therefore chosen to give the detailed results of the study of the relation between total nitrogen of the barleys and the free saccharifying activity (Lintner values) of the malts made from them. The main features of the other relations are summarized in the form of correlation coefficients obtained by means of analyses of variance and covariance.

Correlation Between Total Nitrogen of Barley and Free Saccharifying Activity of Malt

The scatter diagram shown in Fig. 1 contains points for the 144 samples studied. It shows that a definite association exists between the total nitrogen of the barley and the saccharifying activity of the malt made from it, but that

the relation is not particularly close. The correlation coefficient for these data proved to be 0.701, whereas the required value for odds of 99 to 1 that a real relation exists is only about 0.22.

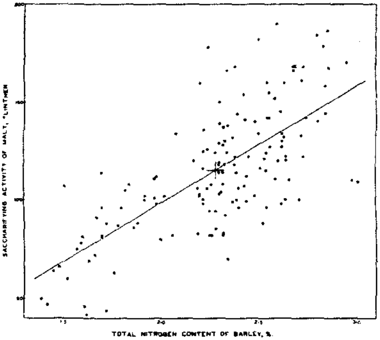

FIG. 1. *Scatter diagram showing the relation between free saccharifying activity (Lintner value) of malt and total nitrogen content of barley.*

More detailed information on the relation is provided by the 12 small scatter diagrams shown in Fig. 2, A to L. Each of these presents the results for one variety only. The graphs show quite clearly that within each variety there is a direct and fairly close correlation between the two variables. It is thus apparent that environmental conditions that produce barleys of high nitrogen content also tend to produce barleys that yield malts of high saccharifying activity. These related effects of environment are illustrated in summary form by the scatter diagram for station means, over all varieties, given in Fig. 2M. The correlation coefficient for the data represented in this graph proved to be 0.961.

A comparison of the small scatter diagrams will show that no inter-varietal relation exists between the two variables. Thus Olli has the highest average saccharifying activity and a low average nitrogen content, but Nobarb with a nitrogen content of about the same value has a comparatively low saccharifying activity. The fact that no inter-varietal relation exists is clearly demonstrated by the scatter diagram for varietal means shown in Fig. 2N. The correlation coefficient for these data is −0.039, which is quite insignificant.

Further inspection of the small scatter diagrams will show that the slopes of the regression lines (regression coefficients), which represent the average increase in malt saccharifying activity per unit increase in barley nitrogen, are not the same for all varieties. The numerical values of the regression

coefficients are as follows: O.A.C. 21, 78; Mensury, 77; Olli, 75; Peatland, 81; Pontiac, 75; Nobarb, 54; Regal, 46; Wisconsin 38, 38; Charlottetown 80, 27; Hannchen, 68; and Victory, 40. In order to determine whether these varietal

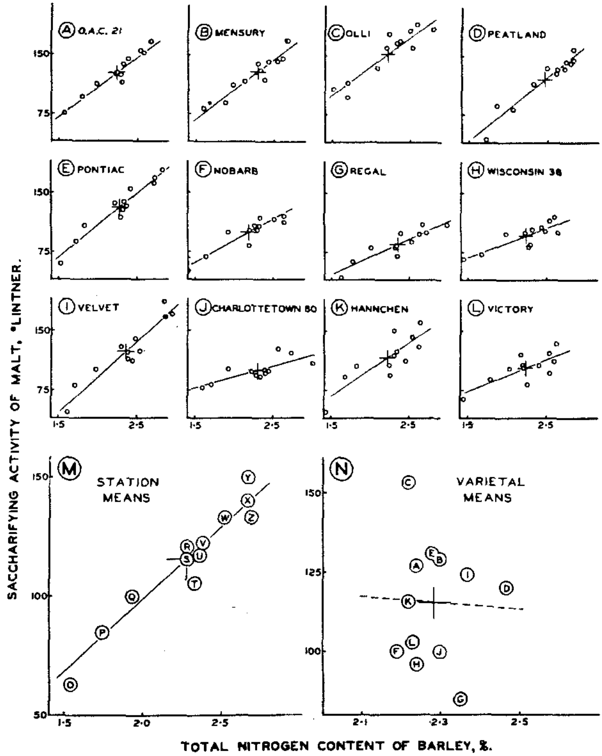

FIG. 2. *Scatter diagrams showing the relation between free saccharifying activity (Lintner value) of malt and total nitrogen content of barley.* A–L. *Diagrams for individual varieties.* M. *Diagram for station means. The key to stations follows:—* O, *Nappan, N.S.;* P, *Fredericton, N.B.;* Q, *Ste. Anne de Bellevue, Que.;* R, *Ste. Anne de la Pocatière, Que.;* S, *Lethbridge, Alta.;* T, *Winnipeg, Man.;* U, *Brandon, Man.;* V, *Guelph, Ont.;* W, *Ottawa, Ont.;* X, *Lacombe, Alta.;* Y, *Beaverlodge, Alta.;* Z, *Gilbert Plains, Man.* N. *Diagram for varietal means. The key to varieties is given in diagrams* A *to* L.

regression coefficients could be considered to differ significantly, the data were subjected to an analysis of residual inter-station, intra-varietal variance. Since the mean square due to differences in varietal regression coefficients (686.5) proved to be significantly greater than that due to deviations from individual regressions (119.0), there is definite evidence that the varietal regression coefficients do differ significantly.

Having completed these detailed examinations of the data, it now seems worth while to reconsider the scatter diagram shown in Fig. 1. It is obvious that this can be built up by superimposing, one upon the other, the 12 diagrams for the individual varieties, shown in Fig. 2, A to L. Considered from this viewpoint, it is easy to see that most of the scatter in Fig. 1 is caused by the fact that there is no inter-varietal relation between the two variables. Moreover, although definite intra-varietal relations exist, the fact that the regression coefficients for these differ also adds to the scatter shown in Fig. 1. However, even if these two sources of scatter were removed, the points would still fail to fall within a really narrow band since the intra-varietal relations are by no means perfect.

With these considerations in mind, it is apparent that relations of the sort now under discussion cannot be adequately elucidated by the study of comparatively small series of samples representing random collections of several different varieties or of mixtures of varieties such as frequently exist in commercial samples. Moreover, when a relation though distinct is not close, it can only be demonstrated when the samples studied provide a wide range of values with respect to each variable. It appears that some of those who have previously investigated the relations between nitrogen content and amylase activity have failed to appreciate these points, or have been unable to collect satisfactory sets of samples for study, and have thus been prevented from obtaining data from which the relations could be demonstrated.

Results of Investigations of Other Correlations and Comparison of These

The results of investigations of all relations between total nitrogen and nitrogen fractions on the one hand, and saccharifying activities on the other, are summarized in Table I. Each relation is represented by four correlation coefficients, namely: the total correlation coefficients, listed in the first section of the table; those for station means, listed in the second section; those for varietal means, listed in the third section; and those for the remainders, listed in the last section.

If space permitted, each of the correlation coefficients in the first three sections of the table could be illustrated by a scatter diagram similar to one of those used to illustrate the relation between total nitrogen and malt saccharifying activity. Those for the total correlations would be similar to the diagram in Fig. 1; those for station means similar to Fig. 2M; and those for varietal means similar to Fig. 2N. The correlation coefficients for remainders do not lend themselves to ready graphical presentation. They represent that

part of the total correlation that is not accounted for by the inter-varietal and inter-station correlations.

The total correlation coefficients given in the first section of Table I are all significant but show differences in the degree of association between the various pairs of variables. A comparison of the data by columns shows the nitrogen and nitrogen fractions are most closely correlated with total barley saccharifying activity and least closely correlated with free barley activity. If the lines of data are compared it will be seen that the degree of association of nitrogen and nitrogen fractions with saccharifying activity is greatest for total nitrogen, not much lower for alcohol-soluble protein nitrogen and total salt-soluble nitrogen, and rather lower for the other three fractions.

The second section of the table gives the correlation coefficients for station means. All these are also significant and somewhat higher than the corresponding coefficients given in the first section. It is thus apparent that a change in environmental conditions that tends, on the average, to increase total nitrogen and nitrogen fractions also tends to increase the saccharifying activities. Again comparing by columns, it is apparent that the correlations between the nitrogen, or nitrogen fractions, and each of the saccharifying activities are of much the same order. A comparison by lines shows that saccharifying activity is most closely associated with total nitrogen content and alcohol-soluble nitrogen, less closely associated with insoluble nitrogen and total salt-soluble nitrogen, and still less closely associated with the other two fractions.

The third section of Table I gives the correlation coefficients for varietal means. These present an entirely different picture. The coefficients for total nitrogen, insoluble nitrogen, and alcohol-soluble nitrogen, on the one hand, and saccharifying activities on the other hand, are not significant. It follows that varieties that tend, on the average, to be higher in total nitrogen and nitrogen fractions of high molecular weight, do not also tend to be high in saccharifying activities. It is apparent that no inter-varietal relation exists between free barley saccharifying activity and total nitrogen or any of the nitrogen fractions. In contrast, it is interesting to note that varieties that contain larger proportions of the more soluble nitrogen fractions also tend to be higher in total barley saccharifying activity and in malt saccharifying activity. The coefficients for the more soluble fractions and latent amylase activity are somewhat lower though still significant.

The last section of the table gives the correlation coefficients for the remainders. These provide further information on the closeness of the various relations studied and serve to substantiate the general conclusions drawn from consideration of the total correlation coefficients and the coefficients for station means.

The individual varietal regression coefficients for several of these relations have been calculated and the significance of the differences between them examined by a statistical method. In each instance significant differences were found to exist, these being of much the same relative order as those

between the varietal coefficients for the regression of malt saccharifying activity on total nitrogen, which were listed in the previous section of the paper. Since the matter is of little theoretical interest and of little practical importance, the detailed data and statistics need not be presented.

TABLE I

ANALYSES OF VARIANCE AND COVARIANCE FOR NITROGEN DATA AND SACCHARIFYING ACTIVITIES

Total nitrogen and nitrogen fractions, % of dry matter	Saccharifying activities, degrees Lintner			
	Malt	Barley		
	Free	Total	Free	Latent
Correlation coefficients, total, $n^{\dagger}$ = 142				
Total nitrogen	.701**	.852**	.643**	.700**
Insoluble protein nitrogen	.520**	.655**	.469**	.548**
Alcohol-soluble protein nitrogen	.635**	.796**	.647**	.615**
Total salt-soluble nitrogen	.676**	.725**	.457**	.645**
Salt-soluble protein nitrogen	.542**	.573**	.362**	.509**
Non-protein nitrogen	.593**	.652**	.409**	.581**
Correlation coefficients for station means, $n^{\dagger}$ = 10				
Total nitrogen	.961**	.976**	.936**	.974**
Insoluble protein nitrogen	.883**	.902**	.791**	.951**
Alcohol-soluble protein nitrogen	.969**	.972**	.962**	.950**
Total salt-soluble nitrogen	.757**	.812**	.779**	.792**
Salt-soluble protein nitrogen	.649*	.658*	.653*	.643*
Non-protein nitrogen	.743**	.838**	.792**	.845**
Correlation coefficients for varietal means, $n^{\dagger}$ = 10				
Total nitrogen	−.039	.198	−.238	.290
Insoluble protein nitrogen	−.239	−.188	−.127	−.076
Alcohol-soluble protein nitrogen	−.324	−.075	−.119	.008
Total salt-soluble nitrogen	.727**	.739**	−.124	.654*
Salt-soluble protein nitrogen	.640*	.660*	−.140	.601*
Non-protein nitrogen	.775**	.770**	−.077	.651*
Correlation coefficients for remainders, $n^{\dagger}$ = 120				
Total nitrogen	.454**	.608**	.461**	.488**
Insoluble protein nitrogen	.290**	.406**	.230**	.376**
Alcohol-soluble protein nitrogen	.321**	.462**	.337**	.382**
Total salt-soluble nitrogen	.275**	.296**	.356**	.142
Salt-soluble protein nitrogen	.156	.187*	.242**	.093
Non-protein nitrogen	.170	.137	.160	.041

NOTE.—*In this and later tables ** denotes that the 1% level, and * that the 5% level of significance is attained.*

† *residual degrees of freedom.*

Some Correlations with 1000-Kernel Weight

Since Bishop's (5) prediction equations for the diastatic activity of malt are based on the relation between diastatic activity and two independent variables, namely, nitrogen content and 1000-kernel weight of the barley, it seemed worth while to investigate this simultaneous relation with our data.

As a first step the relations between total nitrogen and 1000-kernel weight, and between malt saccharifying activity and 1000-kernel weight were examined by analyses of variance and covariance. The resulting correlation coefficients are given in the first two columns of Table II. The total correlations over all samples proved to be significant and negative in both instances. However, it is apparent that neither relation is close and significant correlations were not obtained for varietal and station means, presumably because the number of pairs of values available for study was insufficient. The statistics show, however, that there is a slight tendency for barley samples that are higher in 1000-kernel weight to be lower in total nitrogen content and to yield malts of lower saccharifying activity.

TABLE II

RELATIONS AMONG MALT SACCHARIFYING ACTIVITY (S) AND TOTAL NITROGEN (N) AND 1000-KERNEL WEIGHT (K) OF BARLEY

Correlation coefficients for	r_{NK}	r_{SK}	$R_{S.NK}$	r_{SN}
Total	−.341**	−.391**	.719**	.701**
Station means	−.519	−.434	.964**	.962**
Varietal means	−.552	−.486	.610	−.039
Remainders	.037	−.169	.491**	.454**

Multiple correlation coefficients for the relation between malt saccharifying activity on the one hand, and barley nitrogen and 1000-kernel weight on the other, were then calculated in the usual manner. These are given in the third column of data in Table II, and for purposes of comparison the corresponding coefficients for the simple correlation between malt saccharifying activity and barley nitrogen are given in the last column. The multiple coefficient for varietal means is not significant, and those for all samples, station means, and remainders are only slightly higher than the corresponding simple correlation coefficients for malt saccharifying activity and total nitrogen. It is thus apparent that the prediction of malt saccharifying activity from both total nitrogen and 1000-kernel weight will be only slightly more precise than the prediction from total nitrogen alone.

Since the improvement resulting from the addition of 1000-kernel weight as a second variable was small, it seemed advisable to determine whether it could be considered significant. The variance of the data for malt saccharifying activity was accordingly analyzed into portions accounted for by:— *(i)* total nitrogen, *(ii)* the added effect of 1000-kernel weight, and *(iii)* residual variance. The resulting statistics are given in Table III. For the total,

varietal means, and remainders, the mean squares for the added effects of 1000-kernel weight are significantly greater than the corresponding mean squares for residuals, but the ratio for station means does not attain a significant level.

TABLE III

ANALYSES OF VARIANCE OF MALT SACCHARIFYING ACTIVITY

Variance accounted for by	Total		Station means		Varietal means		Remainders	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Total nitrogen	1	71637**	1	71714**	1	73	1	4142**
Added effect of 1000-kernel weight	1	3796**	1	445	1	17831*	1	695*
Residual	141	499	9	602	9	3355	119	128

As a last step in the study of these relations, the partial correlation between malt saccharifying activity *(S)* and total nitrogen *(N)* independent of 1000-kernel weight *(K)*, and the partial correlation between malt saccharifying activity and 1000-kernel weight independent of total nitrogen were examined. The resulting partial correlation coefficients together with the corresponding simple correlation coefficients are reported in Table IV.

TABLE IV

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS BETWEEN MALT SACCHARIFYING ACTIVITY (S), AND TOTAL NITROGEN (N) AND 1000-KERNEL WEIGHT (K) OF BARLEY

Correlation coefficients for	r_{SN}	$r_{SN.K}$	r_{SK}	$r_{SK.N}$
Total	.701**	.652**	−.391**	−.226**
Station means	.962**	.956**	−.434	.181
Varietal means	−.039	−.422	−.486	−.609*
Remainders	.454**	.467**	−.169	−.209*

A comparison of the statistics in the first two columns shows that 1000-kernel weight has little effect on the relation between malt saccharifying activity and total nitrogen, the correlation coefficients being of essentially the same order, irrespective of whether an adjustment is or is not made for the effect of 1000-kernel weight. On the other hand, a comparison of the last two columns of data shows that total nitrogen has rather more effect on the correlation between malt saccharifying activity and 1000-kernel weight. The partial correlation coefficient for varieties is definitely higher than the correlation coefficient and the former is significant whereas the latter is not. We may therefore conclude that the effect of variations in total nitrogen content tends to mask the relation between malt saccharifying activity and 1000-kernel weight. When an adjustment is made for the effect of total

nitrogen, it becomes apparent that varieties that are lower in 1000-kernel weight tend to be higher in malt saccharifying activity. The other three partial correlation coefficients suggest that the intra-varietal relation between malt saccharifying activity and 1000-kernel weight, independent of the effect of total nitrogen, is almost negligible.

General Discussion

The investigation demonstrates that there is a definite association between total nitrogen content and saccharifying activity and that this relation is closer than that between saccharifying activity and any of the nitrogen fractions studied. When the relation is examined in more detail it is found that it is an intra-varietal relation and does not apply between varieties. This is also true of the relations between saccharifying activity and insoluble protein nitrogen, and between saccharifying activity and alcohol-soluble protein nitrogen. It thus appears that many of the environmental factors that control the amounts of total nitrogen and of the less soluble protein fractions present in the grain are also common to the control of the amounts of amylase elaborated. As a result, environmental conditions that tend to produce high nitrogen barleys also tend to produce barleys that are high in saccharifying activity and produce malts of correspondingly high activity. On the other hand, since no inter-varietal relation exists, it is apparent that few, or possibly none, of the varietal factors that control total nitrogen and the less soluble protein fractions play a part in controlling saccharifying activity.

In contrast to the relations just discussed, those between saccharifying activity and the more soluble nitrogen fractions, though considerably less close, exist both within and between varieties. It thus appears that some of the varietal factors that control the amounts of the more soluble nitrogen fractions present in the grain also play a part in controlling the amount of amylase elaborated in it.

The authors are inclined to accept with reservations the evidence for these inter-varietal relations. It may be that the correlations obtained are merely expressions of differences among the three classes of varieties represented in the investigation, and result only because it so happens that the five rough-awned six-rowed varieties are high in soluble nitrogen fractions and saccharifying activity, whereas the four smooth-awned varieties and the three two-rowed varieties have lower values for these determinations. An examination of a larger number of varieties, containing several representatives of each of several classes of barley, will be required in order to collect sufficient data for an adequate study of these inter-varietal relations.

The relations between total barley saccharifying activity on the one hand, and total nitrogen and nitrogen fractions on the other hand, proved to be slightly higher than the corresponding relations for free malt saccharifying activity. The differences are not great and can probably be accounted for by experimental errors introduced by malting. Latent saccharifying activity, and especially free saccharifying activity, proved to be still less closely asso-

ciated with the nitrogen data. The investigation thus provides no grounds for believing that the distribution of total amylase between the free and latent state is related to the distribution of nitrogen between the various protein fractions.

Since latent saccharifying activity is more closely related to total nitrogen than to any of the nitrogen fractions studied, the investigation fails to provide support for the hypothesis that the latent amylase is rendered unextractable by water through combination with an insoluble protein of high molecular weight. On the other hand, the authors do not consider that the results of the investigation tend to disprove this hypothesis. It may be that a close relation exists between the amounts of latent amylase and of some protein present in the grain, but that this relation is not brought to light because the comparatively crude fractionation methods used fail to isolate the protein in question or any protein fraction with which it is closely correlated.

Although the prediction of malt diastatic activity from data on the total nitrogen content of barley does not appear to be a matter of much practical importance, it seems worth while to consider briefly the bearing of the present investigation on this subject. It is apparent that the prediction equation will be of the type, $y = a + bx$ (a straight line), where y = malt diastatic activity, x = total nitrogen content of barley, and a and b are constants. Since both a and b (the regression coefficient) may differ for different varieties, separate equations will have to be worked out for each variety. In addition, since at least one and probably both constants will be affected by the malting method, particularly kilning, separate sets of equations will have to be developed for different malting conditions. In these circumstances, the prediction equations calculated from the data and statistics discussed in this paper are not given. The investigation appears to yield for general use only a rough rule of thumb, namely, that variety has a comparatively marked effect on diastatic activity, and that within any one variety samples of higher nitrogen content tend to yield malts of higher diastatic activity.

It should also be noted that our study of the simultaneous relation between malt saccharifying activity and the total nitrogen content and 1000-kernel weight of the barley suggests that the very small increase in the precision of prediction that results from the inclusion in the equation of 1000-kernel weight is not likely to compensate for the added work of determining this property.

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A STUDY OF THE MECHANISM OF FROST INJURY TO PLANTS[1]

By D. Siminovitch[2] and G. W. Scarth[3]

Abstract

Observations in a micro-freezing apparatus of isolated tissues of the cortex of hardy and non-hardy plants of *Catalpa* and *Cornus* species, and of the epidermis of red cabbage, reveal that there are two modes of freezing of plant cells, intracellular and extracellular.

In intracellular freezing, ice crystals form first in the protoplasm and then in the vacuole. In extracellular freezing, ice forms outside the cells from water in the cells. The resulting dehydration of the cell causes its collapse, the opposite walls coming together and squeezing the contents to the periphery. Intracellular freezing is fatal to all cells by visible mechanical disruption of the protoplasm and vacuole. It is facilitated by rapid freezing and occurs less easily and less frequently in hardy tissues and in trees and shrubs than in non-hardy and herbaceous tissues. Extracellular freezing induced through slow cooling is fatal to all unhardy cells in trees and herbs at all temperatures below the freezing point, and to cells of hardy cabbage only at −10° C. to −15° C., but not to cells of hardy trees and shrubs.

Both types of ice formation have been observed in intact plants of red cabbage frozen in a refrigerator.

The behavior of hardened plants shows that intracellular freezing tends to be prevented in them by an increased permeability to water. In regard to extracellular freezing, from the behavior of the cells on freezing and in micrurgy, a mechanical injury hypothesis is presented.

Introduction

Because of their hydrated nature, most plants freeze when exposed to temperatures slightly below the freezing point of water. The nature of the tolerance of certain plants to ice formation in winter and the absence of this tolerance in summer have been subjects of intensive investigation by plant physiologists and pathologists in the past century. The study of these problems has been of increasing practical and agricultural importance in temperate climates.

The recent work of Scarth and Levitt (18) has demonstrated that the answer to this problem is to be sought primarily in the study of living cells. It was shown that fundamental changes take place in the protoplasm and vacuole when a plant becomes hardened to frost, changes far exceeding in degree those demonstrated by other methods.

In view of the close correlation of these changes with the hardiness of the plant, these authors postulated a causal relation between the progress of the changes during hardening and the development of resistance to the injurious effects of frost that appear in unhardy or unhardened plants. The nature and location of the changes pointed to mechanical modes of injury, suffered either at the freezing or at the thawing phase, or at both. Indeed, certain types of mechanical injury, from which the hardening changes might afford protection, had already been reported by various authors.

[1] *Manuscript received July 11, 1938.*
Contribution from the Department of Botany, McGill University, Montreal.
[2] *Holder of a Studentship from the National Research Council of Canada.*
[3] *Macdonald Professor of Botany, McGill University, Montreal.*

The first effect of frost injury to be reported was the killing of the plant by formation of ice crystals inside the cells (4, 6, 8, 12, 13, 19). When this occurs, mechanical disintegration of the protoplasm is visible, as will be shown later. With few exceptions (1, 20), intracellular freezing has always been found to be fatal. One of the protoplasmic hardening changes found by Scarth and Levitt is an increase in permeability. They state, "The condition for internal freezing is that the temperature of the cell sap fall below its freezing point. Ice first starts to form on the cell walls outside the cells, where it normally grows at the expense of water that diffuses from cells. If this keeps pace with the fall of temperature, the resulting increase in its concentration will prevent the sap from freezing, but with a sudden drop of temperature or sudden crystallization as a result of super-cooling, the rate at which water can pass out of the cell may be the limiting factor in deciding whether or not ice will penetrate. Here, then, is a condition when high water permeability may mean safety to the cell."

Another mode of mechanical injury occurs at the thawing phase following purely extracellular freezing. According to Iljin (9), the cell wall collapses on freezing, and the cell is killed on thawing by the breaking away of the rapidly expanding cell wall from the more slowly expanding protoplast. The protoplasm is consequently ruptured, either at the moment of disjunction or during the subsequent deplasmolysis phase.

Thus there are at least two modes of mechanical injury that accompany different modes of freezing. The importance of each, and of the cellular change produced during hardening to resist them, depends on the relative frequency of occurrence of the respective modes of freezing. The only instances in the literature (cited above) of observed intracellular ice are restricted to filamentous algae and isolated plant tissues, which were usually exposed to rapid freezing. Also, the locus of ice formation previously observed in intact plants has been intercellular (3, 12, 13, 15–17, 21). These observations have led to the conclusion that the latter is the natural form of freezing and that injury through intracellular ice is abnormal and unlikely to occur in nature. The importance of the mode of thawing as a factor in determining injury has been generally discounted (5, 7, 12, 13). Three authors (5, 12, 13) found a few minor exceptions in which the rate of thawing was of limited importance. Åkerman (1, 2) showed that the rate of thawing was of importance only within a certain temperature range. Iljin did not specify under what conditions he observed the thawing injury or whether it was absent in hardy plants. A third mechanism of injury apparently exists.

Therefore, further investigation is required both as to the frequency and conditions of intracellular freezing and to the effect of extracellular freezing on the cells. Little or no attention has been devoted to observation of differences between the behavior of hardy and unhardy cells on freezing and thawing, and while the hardening changes that Scarth and Levitt reported enabled them to predict certain probable differences in this respect, their inferences remained unverified. Continuing this work, we have made an

intensive study of the visible effects of freezing and thawing on hardy and unhardy cells and tissues, directed along the following lines:

1. A study of the separate behavior of the protoplasm and the vacuole of cells during the freezing and thawing of isolated tissues, and a comparison in this respect of hardy and non-hardy plants.

2. A study of the mode of freezing of intact plants, and a comparison in this respect of hardy and non-hardy plants.

Apparatus and Materials

To make microscopic observations on the natural freezing of whole plants, it was necessary to section the frozen plants and manipulate the sections, without thawing, under the microscope. This was made possible by the use of a refrigerated chamber, artificially lighted and large enough to contain the plant, microscope, and observer. Temperatures of −20° C. could be obtained in this cold chamber, and a thermostat temperature control permitted a uniform drop of 2° C. per hour.

Apparatus was also required whereby isolated plant tissues could be observed continuously under the microscope while the temperature of the environment was altered from that of the room to one considerably below zero and *vice versa*. A certain degree of manipulation of and access to the tissue during exposure to the freezing temperatures was essential. A low-temperature stage, as used by Levitt and Scarth (11, p. 293), forms the basis of the apparatus. In part of the work, low temperatures were attained by placing blocks of solid carbon dioxide or "dry ice" on the exposed metal surface of this stage, on either side of the objective. To facilitate conduction, a non-freezing liquid medium filled the interior of the stage. Temperatures of −20° C. were obtainable in the cell of the stage within ten minutes of application of the "dry ice". In order to prevent frosting, a coating of oil was used on the glass surfaces of the cell exposed to room temperature. A small glass tube surrounding the objective and resting on the metal surface of the stage protected the aperture of the cell, so that the lens of the objective and the tissue were not clouded by condensation of vapor from the carbon dioxide. The tissue was warmed by removing the carbon dioxide and by siphoning a liquid at room temperature through the hollow stage.

The above method gave a constant but uncontrolled rate of drop in temperature which sufficed only for preliminary experiments. To control the rate of fall in temperature for more extensive experiments, cooling was effected later in a different way. A fluid cooled by a freezing mixture (salt and ice) was siphoned through the stage. Control of the rate of flow of the fluid regulated the temperature of the cell.

A galvanometer, calibrated to give the temperature directly, and attached to the junction point of a copper-constantan thermocouple inserted in the cell, indicated the temperatures to which the tissue was being exposed during the experiment.

The plants used, chosen chiefly because their cells lend themselves to microscopic observation, were as follows: woody, *Pyrus malus*, *Catalpa hybrida*, *Cornus* sp.; herbaceous, *Brassica oleracea capitata*.

Experiments and Results

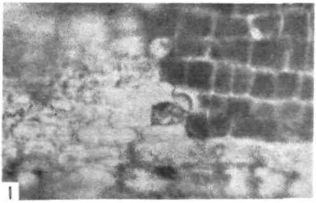
Freezing and Thawing of Isolated Plant Tissues in a Micro-freezing Apparatus

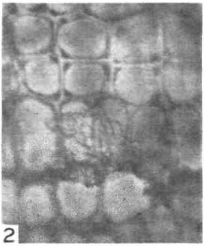
Constant Rapid Cooling by Means of "Dry Ice"

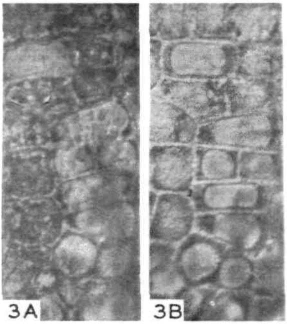
A longitudinal tangential section of the cortex of the twig of a hardy *Catalpa* tree was cut and dried on a filter paper, mounted on a glass disc and covered with oil. The disc was inserted in the cell of the "micro-freezing" apparatus and the blocks of dry ice applied. The temperature in the cell dropped to −12° C. in ten minutes. At that point several protoplasts at one end of the section were "blotted out", or became opaque. In an instant all the protoplasts followed suit, and the section blackened from one end to the other. Closer observation of the phenomenon in individual cells revealed that the opacity was caused by the formation of minute granular ice crystals throughout the protoplasm and vacuole. The ice formed first at one end of the protoplasm, continued around each side of the vacuole to the other end, and finally formed in the vacuole. Each cell froze and darkened similarly, a wave of freezing and darkening passing acrosss the section. A photograph taken as such a wave began is shown in Fig. 1. The first frozen and blackened cells are in the upper right-hand corner.

After the section had thawed, it was removed and centrifuged in a hypertonic solution of calcium chloride and neutral red to determine both by plasmolysis and staining the survival of the cells. All were dead. Naturally-pigmented cortical cells of the twig of a hardy apple tree behaved in exactly the same way. The darkening of the vacuole, however, consequent upon the formation of ice, was intensified by the presence of the pigment; this was used later to indicate the death of the cells. On thawing, the separate character of the protoplasm and vacuole was lost (Fig. 3B), and the pigment diffused out of the cells into the surrounding medium. It was apparent that such freezing within the cell, or intracellular freezing, was fatal.

Similar sections of hardy *Catalpa* trees were subjected to low temperatures in the micro-freezing apparatus, but when the temperature approximated the freezing point the cooled tissue was "inoculated" with a small crystal of ice. (As noted above, the rate of fall in temperature was constant with dry-ice cooling.) As the temperature dropped, the crystal was observed to grow in dimensions and to radiate in all directions over part of the surface of the tissue (Fig. 2). At the same time, needle-like and feathery crystals formed spontaneously at various loci, particularly in the small intercellular spaces between the rounded corners of four cells, but they did not cover the whole area of the tissue. Though held at a temperature of −15° C. for a period of one-half hour, no intracellular freezing took place. It was apparent that the crystals had grown by withdrawal of water from the cells, concentrating the intra-







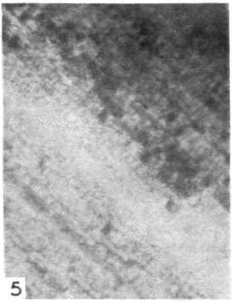


FIG. 1. *Intracellular freezing in cortical tissue of Cornus.* FIG. 2. *Extracellular freezing in cortical tissue of Cornus.* FIG. 3, A. *Hardy tissue of Cornus frozen after supercooling;* B. *After thawing.* FIG. 5. *Contrast between hardy and unhardy tissues of Cornus frozen under water after supercooling. Unhardy tissue, upper right, shows more intracellular ice.*

cellular fluids. This was proved by the behavior of artificially stained cells, similar to that described by Iljin. As the growth of the crystals proceeded, the centres of the cells were observed to become lighter in color until a white patch appeared, surrounded by a brilliant red band at the periphery of the cell. The dehydration of the cells had caused a shrinkage to the extent that the two opposite walls facing the observer had curved inward, come into contact at the centre, and had squeezed the colored vacuole into the periphery of the cell.

Extended observations of this mode of freezing, made on the cortical cells of dogwood *(Cornus)*, showed that as the crystals grew, the protoplasm and chloroplasts shrank and became closely pressed to the cell wall like a thin green film. Fig. 2 shows this condition, as well as the growing and radiating ice crystal. (Reference to Fig. 3B will show the normal thickness of the protoplasm in unfrozen tissue.) It was evident that the protoplasm as well as the vacuole became dehydrated and concentrated during the process. Presumably the freezing points of the protoplasmic and vacuolar fluids were correspondingly lowered, so that they remained below the temperature of the cell as it dropped. Thus only external or extracellular freezing was possible, and when the cells were thawed both the plasmolysis and staining tests indicated 100% survival.

From these observations it is possible to explain the intracellular freezing in the first experiment. The fine capillary structure of the tissues provided ideal conditions for supercooling. Consequently, lacking the stimulus to crystallization afforded by inoculation, the tissue supercooled so far below the freezing point of the cell fluids that when crystallization began, it at once penetrated into all the cells.

In some instances, however, probably because of disturbance, the tissue supercooled only a few degrees below the freezing point. In this event, when intracellular ice formation began, it progressed over the section slowly, appearing gradually in one cell after another. In these preparations it was noticed that certain isolated cells or groups of cells were unfrozen, and remained so, although they were surrounded on all sides by internally frozen cells and were exposed to $-15°$ C. for a period of 20 min. These unfrozen cells were undergoing characteristic dehydration and concentration, as shown by the behavior of the artificially stained *Catalpa* and naturally stained apple twig cells described above. When the section was thawed and subsequently treated with hypertonic solution and neutral red, the dehydrated cells were distincly plasmolyzed and stained.

A particularly clear example of this phenomenon, provided by the cells of *Cornus*, is illustrated in Fig. 3, A and B. Fig. 3A shows the frozen condition of the tissue following supercooling. The internally frozen cells are dark. The lighter cells are dehydrated, and the shrunken protoplasm can be seen as a dark border at the periphery. Fig. 3B shows the swelling of the protoplasm and the survival of the dehydrated cells after thawing, and the disruption and death of the protoplasm of cells that had been frozen internally.

In hardy tree tissues, fatal intracellular freezing occurs when the tissue is supercooled; but extracellular freezing, occurring when the tissue is inoculated with an ice crystal at the freezing point, does not injure the cells. This is clearly demonstrated by Fig. 4, A, B, and C, showing hardy tissue in which both types of freezing occurred at the same time. Fig. 4A shows the section in the frozen condition. The cells with differentiation between the centre and periphery are intracellularly frozen. This differentiation persisted because the original thickness of the protoplasm and vacuole was preserved for the moment by the formation of ice crystals within them, while in the extracellularly frozen or dehydrated cells the protoplasm becomes very thin. Fig. 4B is the same tissue after thawing. Conditions are now reversed. The separate identity of the protoplasm and vacuole of the extracellularly frozen and surviving cells is restored, while in the intracellularly frozen cells it is lost because of the disruption and death of the protoplasm. Fig. 4C illustrates the result of treatment with a hypertonic solution of calcium chloride; only the cells that had frozen extracellularly are plasmolyzed and alive.

Parenthetically, attention is called to the cell indicated by the arrow, in Fig. 4, A, B and C. It is apparent, from the presence of ice crystals in this cell, that it was frozen intracellularly. In the thawed condition it appeared to be alive, and in hypertonic solution even plasmolyzed. The dark band of protoplasm visible at the periphery of the living cells was not as thick or as sharply defined in this cell, nor did it plasmolyze away from the wall as clearly as the others. This is because ice had formed only in the protoplasm, and the vacuolar membrane or tonoplast remained intact. Consequently, this tonoplast plasmolyzed because it retained a certain degree of its semipermeability; but the cell can in no way be considered alive. The protoplasm is destroyed, and the products of its disintegration adhere to the tonoplast, thus accounting for the lack of a clear space between the plasmolyzed part and the cell wall.

In all unhardy tree tissues, fatal intracellular freezing occurred in spite of inoculation. Applied to dry unhardy tissues, an inoculated crystal grew very slowly and radiated only a short distance. As the temperature dropped, intracellular freezing took place over the remainder of the section, internally frozen cells occurring at the very edge of the crystal. Dehydrated cells were limited to the region immediately underlying the crystal. It is to be noted that in hardy tissues, when supercooling occurred before inoculation, intracellular freezing took place but was limited to the cells at the edge of the section and remote from the ice crystal.

Apparently, the explanation of these observations is that the rate of migration of water through the cells to the ice crystal was much slower in unhardy than in hardy cells. That is, the latter were more permeable to water. The cells of unhardy tissue not in contact with the ice crystal could not be dehydrated rapidly enough to lower the freezing point of the cell fluids sufficiently to prevent intracellular freezing. The important factor in these experiments was the rate of passage of water through the cells and tissue, as indicated by

the fact that with supercooling before inoculation, intracellular freezing occurred in cells of hardy tissues remote from the locus of crystal formation and last to be dehydrated. Yet so readily did hardy cells lose water that even when the tissue was not inoculated and intracellular freezing occurred, some cells were dehydrated, if surrounded by others containing ice crystals that attracted water (Fig. 3A).

The importance of the rate of passage of water through the cells and tissues was demonstrated further when unhardy tissues previously immersed in water were frozen. At the freezing point, the water surrounding the tissue froze after inoculation, encrusting the tissue in a flat transparent sheet of ice. Air was liberated at the same time from the intercellular spaces, and extracellular freezing of unhardy tissue, with characteristic dehydration of the cells, occurred. The slight supercooling and the proximity of the ice to every cell made unnecessary a rapid transmission of water through the cells. But, despite the induction of extracellular freezing, unhardy cells were dead on thawing. Thus unhardy cells were not only more susceptible to injury from intracellular ice, but they could also be killed by extracellular freezing.

The behavior of tree cells on thawing indicated that possibly the mode of injury was as described by Iljin in cabbage cells. In hardy cells the protoplasm was observed to swell to several times its dehydrated volume (Fig. 3A and B), while in stained cells the colorless areas in the centre disappeared and the dye was once more uniformly diffused through the vacuole. Apparently, the walls, protoplasm, and vacuole were absorbing water and expanding together to their normal dimensions. In non-hardy tree cells the wall was observed to dilate on thawing as in hardy cells; but at a certain point the protoplast broke away from the wall and seemed to shrink, assuming a "pseudo plasmolyzed" appearance. It then slowly expanded and burst suddenly as it approached the walls, discharging its contents and killing the cell.

Because of the higher permeability of hardy than unhardy cells, they absorbed water more quickly on thawing and were subjected to far greater stresses during deplasmolysis, yet were more resistant than unhardy cells. This may be explained by a difference in "plasticity"*, enabling the hardy cells to resist deplasmolysis more successfully than unhardy, as found by Levitt and Scarth.

In later experiments, the difference in occurrence of intracellular freezing in hardy and non-hardy cells, after inoculation with ice crystals, was not constant. Sometimes the crystals in hardy tissues would not increase in size, and, as the temperature dropped, intracellular ice formed right up to the ice crystals. At other times the ice would spread at once over the whole length of unhardy sections, come into contact with every cell, and prevent

* *The malleability of semi-solid protoplasm by surface tension or other mechanical forces; if the protoplasm were truly liquid, this would be expressed as low viscosity. Since this paper was submitted, Kessler and Ruhland (Planta, 28 : 159-204. 1938) have reasserted that viscosity is increased in hardening. We agree with Scarth and Levitt in regard to the increased plasticity of protoplasm in the hardy condition. The possibility of a parallel change in viscosity in the internal cytoplasm does not affect the argument, if the mechanical injury acts chiefly on the plasma membrane.*

supercooling and intracellular freezing. Thus the non-hardy tissues would show more intracellular ice than the hardy. This may have been due to lack of proper inoculation or to a larger amount of free water in the unhardy tissue. But by previously immersing the section in water (ensuring extracellular ice in contact with every cell), and combining this with different degrees of supercooling before inoculation, it was possible to demonstrate the differences in permeability between hardy and unhardy plants of the same species and between tissues of different species. Table I shows the results for a series of such experiments, using both hardy and non-hardy tissues of *Catalpa* and *Cornus*.

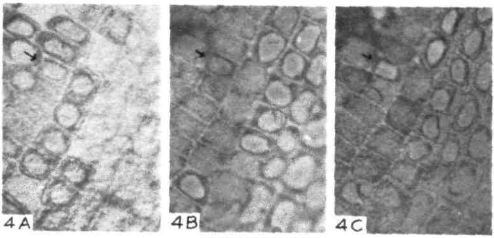
TABLE I

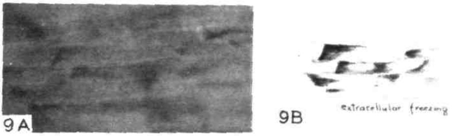
PERCENTAGE OCCURRENCE OF INTRACELLULAR FREEZING IN CORTICAL SECTIONS OF *Catalpa* AND *Cornus* TREES FROZEN UNDER WATER WITH VARIOUS DEGREES OF SUPERCOOLING. RATE OF FALL IN TEMPERATURE, 2° C. PER MIN.

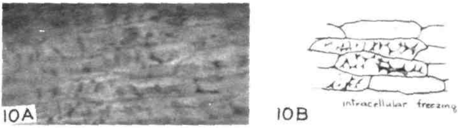
Temperature at inoculation	*Catalpa*		*Cornus*	
	Unhardy	Hardy	Unhardy	Hardy
−2° → −3° C.		5		
	75	0	25	0
	75	25	50	0
	75	25	5	0
−3° → −4° C.	80	25	5	0
	75	50	25	0
	100	10		
	100	10		
	100	50		
−4° → −5° C.	100	50	25	0
	100	50	50	0
	100	75	25	
	100	50		
−5° → −6° C.			90	10

It is evident that the hardier the plant the greater is its resistance to intracellular freezing, and also that *Cornus* is more resistant than *Catalpa*. This difference is shown in Fig. 5, in which a hardy and an unhardy *Cornus* section were frozen side by side. The dark cells are intracellularly frozen and are present to a greater extent in the unhardy section. Unhardy *Cornus* seems more resistant than hardy *Catalpa* to this mode of injury. This would imply a contradiction but for the fact that other kinds of injury were found, such as that sustained in all unhardy cells as a result of extracellular freezing and to which hardy tree cells are totally resistant.

It was impossible to produce extracellular freezing of herbaceous plants, even of hardy tissues, at the rate of freezing obtained with dry ice. Their susceptibility to intracellular freezing may be ascribed to the properties of their cells, which are large, with little colloidal matter and sap of low concentration. As these cells have a low ratio of surface to volume, a relatively large amount of water must be lost to raise the concentration appreciably, so that the lowering of the freezing point of herbaceous cells cannot keep pace with the rapid drop in temperature produced by solid carbon dioxide. It was possible that slower freezing would produce extracellular ice in hardy or non-hardy herbaceous tissues. By use of the apparatus already described, slower freezing with temperature control was obtained. By this means it was possible to show that the same difference exists between hardy and non-hardy tissues of herbs as was shown in trees.







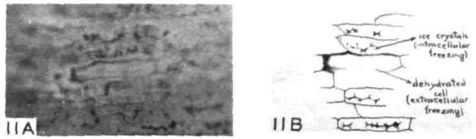


FIG. 4. A. *Intra- and extracellular ice in cortical cells of Cornus;* B. *After thawing;* C. *In hypertonic calcium chloride.* FIG. 9. A. *Extracellular ice in leaf of red cabbage;* B. *Diagram.* FIG. 10. A. *Intracellular ice in leaf of red cabbage;* B. *Diagram.* FIG. 11. A. *Intra- and extracellular ice occurring together in leaf of red cabbage;* B. *Diagram.*

Rate of Cooling Controlled and Varied by Regulating the Flow of Cooling Fluid

In this and subsequent experiments on herbaceous plants, red cabbage was used. It can develop a fair measure of hardiness, which permits of a comparison between hardy and non-hardy plants, and its colored cells lend themselves to observation of intracellular freezing, both in isolated tissues and in the intact plant.

The first attempt was to slowly freeze sections of the petiole of an unhardy and a hardy cabbage in order to obtain extracellular formation of ice. This was effected by careful regulation of the flow of cooling fluid and inoculation at 0° C. Extracellular freezing is characterized in these red-stained cells by whitening at the centre of the cell and by darkening at the periphery (Fig. 11B). The sections were taken directly from the leaf and placed under oil without previous immersion in water. Sap escaping from cut cells resulted in the formation of ice, after inoculation, throughout the section and in

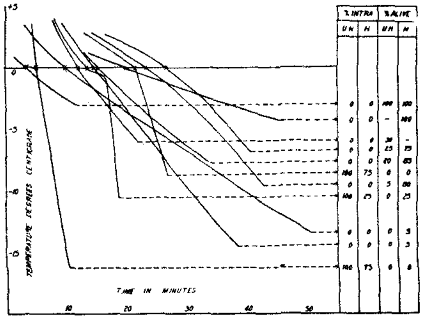


FIG. 6. *Effect of rate and degree of fall in temperature in the freezing of epidermis of red cabbage.* U.H. = *unhardy;* H = *hardy;* X = *point of inoculation with ice crystal; Intra = intracellular ice. Ice extracellular unless otherwise indicated.*

contact with every cell, which prevented localized supercooling. Uniform supercooling at a desired temperature was made possible by postponing the inoculation until this temperature was reached. When such supercooling of the tissue was carried below a certain temperature, intracellular freezing always occurred in both hardy and non-hardy cells. As with rapid cooling, "flashes" of ice formation within the cells were visible all over the section, but only at the moment of inoculation. At the same time the pigment in these cells distributed itself in an irregular but characteristic pattern, with intensification of color. The ice crystals within the cells had displaced and concentrated the pigment. This appearance, combined with the "flashes",

was a certain indication of intracellular ice formation, and was used subsequently for its recognition (Fig. 11B). As in tree tissues, its occurrence always proved fatal to both hardy and unhardy tissues.

A series of experiments on hardy and unhardy tissues, in which the degree of supercooling and the rate and degree of drop in temperature were varied over a wide range, was now carried out. The results of these experiments are represented in Figs. 6 and 7. Fig. 6 shows the results with variation in rate and degree of drop in temperature. Fig. 7 shows the results with different degrees of supercooling. Each line represents the temperature in the cell of the cold stage during the simultaneous subjection of two tissues, one hardy and the other unhardy, to temperatures below the freezing point. The time of inoculation, and therefore of inception of ice formation, is indicated by X. From the lowest point of each graph, which represents the lowest temperature reached for the two tissues concerned, a broken line is drawn to the record of the percentage survival and the percentage of intracellular ice in the cells of the two tissues.

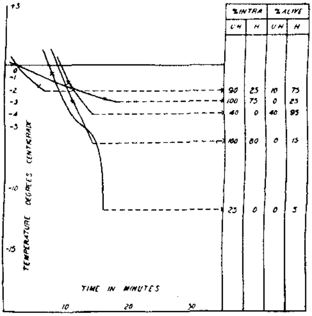


FIG. 7. *Effect of supercooling in the freezing of epidermis of red cabbage.* U.H. = *unhardy;* H = *hard;* X = *point of inoculation with ice crystal; Intra = intracellular ice. Ice extracellular unless otherwise indicated.*

Figs. 6 and 7 indicate that (i) formation of intracellular ice is induced by rapid freezing resulting from rapid drop in temperature or from supercooling previous to inoculation; although it is equally fatal to hardy and non-hardy cells, it occurs more readily in the non-hardy; (ii) extracellular freezing is induced through slow cooling and prevention of any marked supercooling. It is fatal to unhardy cells at all temperatures below the freezing point, while hardy cells are immune above -8 or $-9°$ C. Below this temperature, hardy cells also succumb.

It is apparent that while hardy herbaceous cells can be killed by extracellular and by intracellular freezing, their resistance to both is greater than that of unhardy cells. This resistance to intracellular freezing may be explained by a greater permeability to water. No explanation is offered for the greater resistance shown to extracellular freezing by hardy cells. The injury on thawing seen in tree cells was not observed in the cabbage cells.

Establishment of the role and importance of permeability in frost resistance depends, however, on a demonstration of intracellular freezing as a mode of injury occurring in nature. We therefore extended our observations to whole plants exposed to normal rates of fall in temperature.

Freezing of Whole Plants and Leaves by Slow Cooling in a Refrigerator

Several leaves from an unhardy cabbage plant at room temperature were placed in the refrigerator at $-2°$ C., following which the temperature of the refrigerator was lowered. By means of a thermocouple inserted into the petiole of one of the leaves, and connected to a galvanometer outside the refrigerator, a temperature record of the leaf tissue was obtained. The air temperature of the refrigerator was read through a window, from a thermometer. The change of the temperature of the leaf as well as that of the refrigerator are shown in the upper graph of Fig. 8.

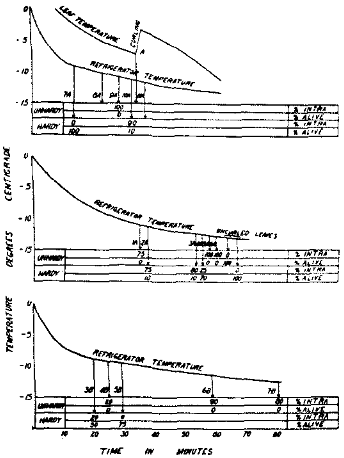


Fig. 8. *Effect of freezing on leaves and whole plants of hardy and unhardy cabbage. Intra = intracellular ice.*

During the series of temperature readings, a sudden deflection of the galvanometer, in a one-second interval, indicated an abrupt rise in leaf temperature (Fig. 8, point A). Direct observation revealed that this leaf was curling, and that some of the others were already curled. On examination, it was found that the curled leaves were brittle and discolored, the uncurled leaves were pliable and of normal color. The former were apparently frozen, the latter were not. This was proved to be the case when all leaves were thawed immediately; the curled leaves became flaccid, the remainder were still

turgid. They were placed in water for a few days and examined. The frozen leaves were now decaying, and the pigment had diffused from their cells, while the unfrozen leaves, which had not curled, were healthy in appearance, turgid, and colored. This showed that the cells of the curled leaves had been frozen and killed while the others had not. A useful criterion of the inception of ice formation was established in this way. Hardy leaves behaved in exactly the same way, except that some of the cells of the curled leaves were not killed. Nevertheless the brittleness, the change in color, and the appearance on microscopic observation of the leaves gave sufficient evidence of freezing of the curled leaves.

Fig. 8 shows that the leaves of cabbage supercool to approximately $-7°$ C. when sudden formation of the ice with evolution of heat occurs. The temperature then falls slowly, representing the slow approach of the temperature of the leaf to equilibrium with the temperature of the surrounding air. It is clear also that some leaves supercooled much more than others, as indicated by the later incidence of curling. Sometimes an hour would elapse between the freezing of one leaf and another.

When whole cabbages were used, all the leaves froze at approximately the same time, indicating that they supercooled to about the same extent. Differences in degree of supercooling as great as or greater than those shown between detached leaves were apparent between whole plants.

Because intracellular freezing always accompanied supercooling of isolated tissues, it might be expected that this would occur in whole leaves and plants. To investigate this point, a number of hardy and unhardy leaves and plants of red cabbage were placed in the refrigerator, and the temperature was lowered. A simultaneous record of time, air temperature in the refrigerator, and curling of each leaf was made by means of the observation window. When most of the leaves had frozen, the uncurled leaves were removed and the curled leaves were examined as quickly as possible, under a microscope previously placed in the refrigerator.

The ready visibility of the red parenchyma cells along the veins, where they are unobscured by green chloroplasts, and their characteristic appearance when frozen made the presence of intracellular freezing quickly evident, and easily distinguishable from extracellular freezing, in which the centre of each cell whitens. The relative number of cells showing intra- and extracellular freezing was recorded for each leaf. Then the leaves were thawed and placed in water. At this time and also a few days later they were examined for viability by the criterion already described. In the study of whole plants, the leaves were removed and examined in the refrigerator as each one froze, so that the combined results represent the frozen plant as a whole. All frozen leaves and plants were exposed to the lowest temperature shown on their respective graphs, regardless of their freezing temperatures.

Fig. 8 shows the results of two freezing experiments on whole leaves and one on whole plants. The temperature in the refrigerator during the three experiments is represented by the curves. At various points along each

curve, arrows indicate the time and temperature at which freezing of each leaf or plant occurred. The percentage of cells containing intracellular ice and of those surviving is shown below each arrow. Observations on the uncurled leaves removed from the refrigerator are shown on one curve. These were unchanged in color and not frozen.

Surface cells in the veins of frozen leaves were photographed in the refrigerator to illustrate the contrast in appearance of intra- and extracellular freezing. Fig. 9A shows extracellular freezing in Leaf 7A; the pigment is concentrated between the cell protoplasm and the vacuole. Fig. 10A shows intracellular freezing in Leaf 10A, which froze later than Leaf 7A, and was therefore exposed to a lower temperature (Fig. 8, top); the pigment is concentrated irregularly between the ice crystals. Fig. 11A shows both types of freezing occurring in one leaf (Leaf 2A, Fig. 8, centre). In the centre of the photograph can be seen an extracellularly frozen and dehydrated cell, with concentration of color at the periphery. It is distinct from the surrounding intracellularly frozen cells, in which the color is concentrated irregularly between the crystals. Diagrams of the two types of freezing are shown in Figs. 9B, 10B, and 11B.

On thawing, leaves that had undergone partial intracellular freezing showed red patches. Under the microscope these patches revealed red living cells distributed over the field and standing out sharply from the dead colorless cells that had been frozen intracellularly.

While the results presented in the graphs are not sufficiently numerous to permit of generalization, they indicate that intracellular freezing may endanger both hardy and non-hardy plants in nature. It was the only mode of injury of hardy cabbages at the temperatures of these experiments (Leaf 3A, 4A, Fig. 8). While the rates of temperature decrease may have exceeded those occurring in nature, the rapid drop at the beginning of two experiments did not affect the production of intracellular ice. The determining factor in its formation is the extent and not the rate of supercooling. When supercooling is slight, extracellular freezing predominates and the injury to hardy cabbage is slight (*e.g.*, compare Leaves 7A and 10A, Fig. 8).

Since unhardy cabbages supercool in the same way, they also undergo and are killed by intracellular freezing, the extent of the latter varying with the degree of supercooling (compare Plants 4B and 6B). The results do not show whether unhardy cabbages are more susceptible than hardy ones to intracellular freezing, with one exception (compare 4B and 5B). The results with whole plants corroborate the results with tissue in that unhardy individuals can be killed by extracellular freezing alone.

Discussion

It is clear that intracellular freezing is a natural and important source of injury by frost. Indeed, it is likely that the death of herbaceous plants at early frost can be ascribed solely to this type of freezing, because when hardened they can survive extracellular freezing at temperatures then obtaining.

When not hardened, either type of freezing kills them. In direct contrast to these herbaceous plants are the hardy trees and shrubs that never succumb to frost under any natural conditions, because they never undergo intracellular freezing and are immune at all temperatures to the effects of extracellular freezing.

Thus there are two mechanisms involved in the development of hardiness or of resistance to frost injury. The first is prevention of intracellular freezing, the second is resistance to injury from extracellular freezing. This is shown most clearly in the experiments with isolated tissues taken from plants that can develop hardiness. They display both types of resistance to freezing to a much higher degree in the hardened than in the unhardened condition. Therefore, complete hardiness seems to imply the successful operation of both mechanisms of resistance.

Intracellular freezing is apparently avoided by increase in the permeability of the protoplasm to water. Such an increase shows strong correlation with the degree of hardiness of a plant, as found by Levitt and Scarth, and can be deduced from the difference in behavior on freezing of hardy and non-hardy tissues. It is apparent that cell permeability to water is an important factor in hardiness; its measurement should be of use in estimating this property.

The mechanism of resistance to injury from extracellular freezing is still obscure. In some instances mechanical injury was observed at the thawing phase, but this occurred only in tree tissues and was associated with extremely rapid thawing (9). The greater resistance of hardened cells of trees to this type of injury may be due to lower viscosity of their protoplasm; but cells of tender or unhardened plants can be killed by extracellular freezing, even though the injury is not apparent during thawing.

Slow thawing rarely saves a frozen plant that would otherwise perish. Similarly, cells dehydrated by evaporation (which produces morphological effects similar to freezing) can be allowed to reimbibe moisture very slowly, yet if they are desiccated beyond a critical point that depends on their drought hardiness, they do not survive (Whiteside, unpublished results). But dehydration *per se* does not appear to be the injurious factor, because when produced by strong plasmolysis it is not necessarily fatal to even the least hardy cells. In fact, the chief cause of death after plasmolysis is the mechanical injury that may occur during deplasmolysis, if the latter is too rapid. The inference is that dehydration by frost also produces injury through some of its mechanical effects. Considering the drastic alteration in shape and volume that a cell undergoes on dehydration, it is not surprising if some of its essential organization is destroyed or incapable of repair when the cell starts to reimbibe water.

Micrurgical study has shown that repair of the plasma membrane is unlikely when its destruction is rapid, and especially when the protoplasm is highly viscous. This condition obtains when a strongly dehydrated protoplast starts to imbibe water. The lower viscosity of the protoplasm of hardy cells facilitates the reorganization of the plasma membrane. By continuing

this study through micrurgical experiments and by comparison with true drought resistance, we hope to examine further what is probably the commonest mechanism of frost injury, the effect of extracellular freezing.

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FRACTIONAL SOLUBILITY OF GLUTEN IN SODIUM SALICYLATE SOLUTIONS[1]

BY E. Y. SPENCER[2] AND A. G. MCCALLA[3]

Abstract

The amount of gluten protein dispersed by sodium salicylate solution is *proportional to the concentration of the salt, while the completeness of extraction* with any concentration depends on the penetration of the salicylate solution into the gluten particles. The higher concentrations (5 to 10%) penetrate rapidly because they disperse most of the protein and automatically expose fresh surface for attack. The lower concentrations penetrate slowly unless the non-dispersed protein is occasionally removed by vigorous stirring or shaking. The amount of protein in dispersed form in any specific concentration of salicylate is the same whether determined by extraction or by dilution of a dispersion in 8% salicylate.

The amide nitrogen of protein fractions obtained by fractional solubility is the same as that of corresponding fractions obtained by precipitation. Results confirm an earlier conclusion that, except for the most soluble 15%, gluten protein consists of a single complex that can be progressively fractionated.

Introduction

Studies on the fractionation of gluten dispersed in sodium salicylate solutions (12) led to the conclusion that the major part of the protein in gluten forms a single complex that can be reversibly fractionated into an indefinite number of component parts differing systematically in physical and chemical properties. Since these results were published there have appeared several papers (2–5) in which the authors reach conclusions at variance with this. The most important of these from our point of view is that by Blish (2), in which the results of extensive experiments employing both fractional precipitation and fractional solubility methods are discussed. The results obtained using the former supported the conclusions of McCalla and Rose, while those obtained using the latter did not. An independent consideration of Blish's results is impossible as no experimental data were published. It is sufficient at this point to note that Blish favors the hypothesis that flour proteins are constituted of relatively few components or component groups.

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[2] *Graduate Assistant.*

[3] *Research Assistant, Associate Committee on Grain Research, University of Alberta, Edmonton.*

He considers that fractional precipitation methods have not furnished trustworthy and conclusive evidence regarding the number of protein fractions. Further discussion of these conclusions is only possible after presentation of the results of the present study.

Harris and Bailey (5) and Harris (3, 4) also favor the hypothesis of few protein components, although the published results have all been obtained from studies in which the isolation of relatively few fractions was attempted. They state that alterations in the conditions of fractionation resulted in different proportions of the three groups obtained. This in itself is definite evidence against the individuality of the fractions obtained, but since fractional precipitation methods were used, these results are open to the general criticisms outlined by Blish.

Harris used the method of preparing and fractionating dispersions in sodium salicylate as outlined by McCalla and Rose (12). He encountered difficulty in securing concordant replicate results when magnesium sulphate solution was added to the dispersions. Since Harris was primarily interested in the relations existing between the quantities of the various fractions and wheat quality, this study yielded little new information regarding the fundamental nature of the protein. Nevertheless Harris is inclined to believe that the fractions obtained are thrown down as mixtures containing varying proportions of each of the few components.

Rich (13) made a study of the dispersion of flour protein in various salt solutions. His results support the conclusion that there are no clear-cut borders in the solubility of flour proteins.

Further evidence has been presented in support of the conclusion that so-called "gliadin" is itself a component system. Krejci and Svedberg (7) found by means of ultracentrifugal determinations that gliadin as extracted by 64% alcohol is heterogeneous as to molecular weight. The least soluble fraction contained a high concentration of heavy molecules, while the most soluble fraction consisted almost entirely of the constituent of lowest molecular weight. Lamm and Polson (10) studied similar gliadin fractions by means of diffusion constants obtained with a refractometric method. All but the most soluble fraction proved to be non-uniform. Kuhlmann (9) studied gluten using alcohol solutions of various concentrations, and concluded that gliadin is not a chemical individual. He suggests that the length of the micelles of the various gliadin components vary, and that glutenin is made up of micelles which are longer than those found in gliadin.

Krejci and Svedberg (8) have further demonstrated that the protein fractions extracted from wheat flour with each of the halides of potassium are mixtures of proteins, and that the mean molecular weight increases from that of the mixture extracted with the fluoride to that of the mixture extracted with the iodide.

The results of a study (11) made on the nitrogen of developing wheat grain support the protein complex hypothesis. These results indicated that the non-protein nitrogen present in the wheat kernel at any definite stage of devel-

opment was the precursor of a specific fraction of the gluten protein. The regularity of the changes in composition of the non-protein nitrogen fraction, and the subsequent formation of corresponding protein fractions make it appear unlikely that there would be any sharp break in the properties of the successive gluten protein fractions.

The work discussed in this paper was undertaken with the object of determining the validity of the conclusions reached as a result of fractional precipitation studies. It was recognized that Blish's criticisms of fractional precipitation methods were justified if there were at least three protein components in gluten. The results presented in this paper were therefore obtained using fractional solubility methods.

Materials and Methods

All experiments reported in this paper were carried out using an unbleached flour milled from high quality, sound Reward wheat.

Gluten was washed from the flour and dispersed in essentially the manner described by McCalla and Rose (12). The principal change in the procedure finally adopted was the introduction of a mechanical stirrer, instead of shaking by hand the flasks containing gluten and solution. The ball of washed gluten was divided into pieces approximately 3 to 5 mm. in diameter, placed in 200 cc. of solution in a 500-cc. wide-mouthed Erlenmeyer flask, and stirred for five minutes.* The flask was set aside at room temperature for 22 hr., and then the contents were stirred for 15 min. The non-dispersed fraction was removed by centrifuging at 2500 r.p.m. for 20 min. The liquid was decanted, and the non-dispersed protein washed twice with sodium salicylate solution of the same concentration as that used in dispersion. When gluten was re-extracted with a second lot of solution, the procedure was repeated.

Concentrations of sodium salicylate from 0 to 10% were used, and all solutions were saturated with toluene. This was essential to prevent biological activity in water, and was used throughout for the sake of uniformity. In all but one experiment, the salicylate used was from a single lot.

Total nitrogen was determined by the Kjeldahl method, using mercuric oxide as catalyst. Hydrolysis and analysis of the gluten fractions followed the methods used in the earlier work (12).

Results

Fractional solubility of gluten

The amount of gluten nitrogen dispersed by sodium salicylate solutions had been found to be roughly proportional to the concentration of the solution (12). As it seemed possible that in the lower salicylate concentrations the extraction of dispersible nitrogen was incomplete, this experiment using four concentrations of sodium salicylate was repeated. Instead of a single extraction being made, each gluten sample was extracted four times, the dispersed

* *Since this study was made, it has been found that it is better to omit the initial stirring. As dispersion depends on penetration of the gluten by the solution, little or no dispersion takes place when the gluten particles are stirred immediately after being placed in the salicylate.*

protein being removed after each extraction. The results are shown in Table I, each figure being the mean of duplicate results. The duplicate values given are for parallel experiments conducted several weeks apart.

TABLE I

PERCENTAGE OF TOTAL GLUTEN NITROGEN DISPERSED BY FOUR SUCCESSIVE EXTRACTIONS WITH SODIUM SALICYLATE

Extraction No.	Concentration of sodium salicylate, %							
	0		2		5		8	
	a	b	a	b	a	b	a	b
1	5.5	5.7	34.1	38.5	70.7	70.1	96.8	96.5
2	7.0	3.5	6.7	6.8	3.2	4.2	0.5	0.8
3	2.5	2.0	4.0	2.6	1.1	1.2	0.3	0.2
4	3.6	1.8	2.1	1.7	0.6	0.6	0.1	0.0
Total	18.6	13.0	46.9	49.6	75.6	76.1	97.7	97.5

There is good agreement between the results for the two experiments except where water was used as the dispersing agent. The first extraction was most effective with the 8% sodium salicylate, and progressively less effective as the concentration decreased. While negligible amounts were dispersed by the fourth extraction with 8 and 5% salicylate, it seemed evident that there was still incomplete dispersion by 2% and water. The agreement between the results of the two experiments with water was relatively poor. The reason for this is discussed later.

The effect of 2% sodium salicylate over a more prolonged extraction period was then investigated. Samples of gluten were extracted for nine successive days and the dispersed nitrogen was removed after each extraction. The results are given in Fig. 1. These demonstrate conclusively that the dispersed nitrogen approaches a limit, which in this experiment was approximately 52% of the total gluten nitrogen. At the same time a sample of gluten was dispersed in 8% sodium salicylate, and the dispersion diluted to 2% salicylate. The amount of nitrogen remaining in dispersed form was 51% of the total, which is in excellent agreement with the total dispersed with repeated extraction of the gluten.

As it had been found that repeated extraction was necessary to effect complete removal of the gluten nitrogen dispersible by the lower concentrations of salicylate, and that the amount of nitrogen so dispersed in 2% salicylate was the same as the amount remaining dispersed after dilution of the salicylate from 8 to 2%, it was decided to compare the values obtained using the two methods over the whole range of unit concentrations from 1 to 8%. Dispersions were stirred for four successive days, but the dispersed material was not removed until after the fourth day. Preliminary tests had shown that it was the exposure of fresh surface, and not the removal of dispersed nitrogen,

that led to increased dispersion with repeated extraction. At the same time a stock dispersion in 8% sodium salicylate was prepared. Aliquots of this were diluted to give the desired concentration of sodium salicylate, and to give the same concentration of nitrogen as in the individual dispersions.

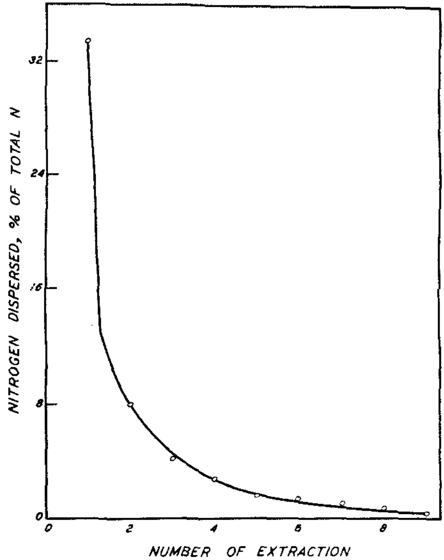

FIG. 1. *Nitrogen dispersed on repeated extraction of gluten with 2% sodium salicylate.*

Dispersed and non-dispersed fractions were separated by centrifuging. The results are given in Fig. 2. There is excellent agreement over the whole range of concentration, with the amounts obtained by dilution being slightly higher than those obtained by dispersion. This was to be expected, in the lower concentrations at least, as dispersion is not quite complete even after four successive extractions.

The amounts of nitrogen dispersed by the two methods are much lower than for the corresponding concentrations of sodium salicylate in Table I. All the earlier experiments had been carried out with C.P. grade salicylate, but in this particular experiment U.S.P. grade was used. As this offered

the only reasonable explanation for the differences, the experiment was repeated with a new lot of C. P. grade salicylate in concentrations of 2, 5 and 8%. The results are given in Table II and Fig. 2. These values are considerably higher than for the U.S.P. salicylate, but lower for the 2 and 5% concentrations than those in Table I. Investigation showed that the differences in dispersing power were related to differences in pH of the salicylate solutions, and to salt impurities. This phase of the problem is at present under investigation, as the standardization of salicylate solutions is essential if concordant results are to be obtained.

TABLE II

PERCENTAGE OF TOTAL GLUTEN NITROGEN DISPERSED BY FOUR SUCCESSIVE EXTRACTIONS, AND REMAINING DISPERSED AFTER DILUTION FROM A CONCENTRATED SOLUTION, C.P. SALICYLATE

Method	Concentration of sodium salicylate, %		
	2	5	8
Dispersion	36.7	69.6	97.6
Dilution	40.3	71.3	97.8

That time of exposure to sodium salicylate solutions is not the main factor in the dispersion of gluten nitrogen is shown by the results in Table III. Glutens were prepared as usual, but after the initial stirring were set aside for periods of 1, 3, 6 and 9 days. Dispersion in 8% solution was almost complete at the end of one day, a result in agreement with those in Table I. Dispersion in 5% was slightly less at the end of nine days than it was after four successive extractions, although the gluten had been exposed for more than twice as long. This effect was much more marked with the samples in 2% salicylate. The amount of nitrogen dispersed after nine days' exposure *without stirring*, except at the end of the exposure, was 10% less than after four successive extractions, and about 15% less than the total amount extractable with 2% salicylate. Thus the removal of the non-dispersed gluten and the exposure of fresh surface is required before complete extraction of the specific protein fractions soluble in the lower salicylate concentrations can be secured.

TABLE III

PERCENTAGE OF TOTAL GLUTEN NITROGEN DISPERSED BY SODIUM SALICYLATE AFTER EXPOSURE FOR DIFFERENT LENGTHS OF TIME

Time of exposure, days	Concentration of sodium salicylate, %			
	0	2	5	8
1	5.7	33.5	70.1	96.7
3	15.3	34.7	71.3	96.2
6	35.4	36.1	73.5	95.7
9	41.7	37.3	75.3	97.5

The behavior of the gluten in water was abnormal. Although the water was saturated with toluene, some factor other than the normal dispersing action was effective in increasing the amount of nitrogen dispersed. This factor appeared to be inhibited in sodium salicylate solutions. It seems likely that the effect was brought about by a disaggregating enzyme of the type discussed by Blagoveschenski and Yurgenson (1). This is indicated

by the results of analyses of the protein dispersed. When only a short extraction period is used, the water-soluble protein is low in amide and high in arginine nitrogen. With extraction periods such as were used in this experiment, however, the amide and arginine percentages approach those of the whole gluten, indicating that there is disaggregation of the gluten as a whole. This phase of the work has not been continued, but obviously water should not be used as a dispersing agent for gluten if the experiment is to be carried over any appreciable period of time.

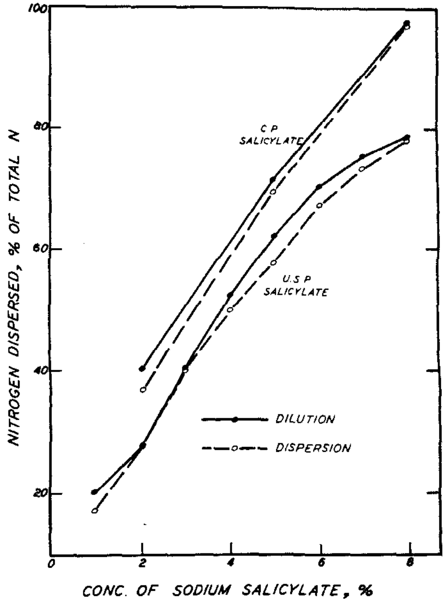

FIG. 2. *Comparison of the amount of gluten dispersed by repeated extraction, and by dilution of a complete dispersion in 8% sodium salicylate.*

The results of extraction and precipitation experiments support the hypothesis that gluten protein is made of a large number of components. In the fractional precipitation studies, however, individual fractions representing only small portions of the protein had been isolated and analyzed.

Attempts to obtain similar small but definite fractions by solubility methods met with comparatively little success. The results, however, are important in the consideration of the methods and of the apparent discrepancies between results obtained with them and with precipitation methods (2). Briefly summarized, these results are as follows:

If gluten is extracted a sufficient number of times to remove most of a fraction of specific solubility, considerable denaturation of the remaining protein results. This is shown by the fact that four extractions of gluten with 1% and re-extraction with 2% sodium salicylate failed to remove as much total protein as a single extraction with the 2% salicylate. Likewise when gluten was extracted four times with 1% salicylate, complete dispersion of the remainder in 10% could not be accomplished. The amount remaining undispersed was 14.5%, although after a single extraction of gluten with 10% salicylate, only 2 to 3% remained undispersed.

The mechanical stirring of the gluten and solution appears to be the cause of the denaturation. Continuous stirring or shaking under similar conditions dispersed less protein than did a single extraction as used in this study. Thus there are two opposing factors effective in determining the amount of protein that can be dispersed in any specific concentration of salicylate. It is necessary to use such mechanical means as stirring or shaking in order to remove the non-dispersed fraction from the unextracted portions of the gluten, but *the greater the mechanical agitation, the greater the denaturation.* The critical factor in the dispersion of any specific gluten fraction is the penetration of the gluten by the salicylate solution. The higher concentrations of sodium salicylate penetrate rapidly because they disperse most of the protein and hence automatically expose fresh surface. Probably nearly complete extraction of any specific protein fraction could be effected in much less time than is now necessary, if the right combination of frequency and degree of agitation could be determined.

Finally, this denaturation results in overlapping of solubilities of otherwise distinct fractions. Part of the denatured protein is removed when the gluten is re-extracted with a higher concentration of salicylate, and contaminates the main portion of this fraction. How important this factor may have been in Blish's (2) attempts to isolate individual fractions with definite properties it is impossible to say, but it certainly cannot be ignored.

The results obtained by Rich (13) were so contrary to those discussed here that his experiment with sodium salicylate was repeated. He reported that 4% ($N/4$) salicylate removed 85% of the protein from flour with one extraction and 100% with two. Our results show only 63% dispersed with two extractions. This is a smaller proportion of the gluten nitrogen than is dispersed when washed gluten is used. Furthermore, 8% salicylate dispersed only 75% of the flour nitrogen and caused marked gelatinization of the starch. The only explanation that can be offered for the results obtained by Rich is that he apparently did not use a centrifuge, and so his "dispersion" may have been, to a considerable extent, a suspension. This is not unlikely, as gluten

protein that appears to be completely dispersed may yield considerable solid matter when centrifuged in an ordinary centrifuge, and still more when a supercentrifuge is used. Differences in pH of different salicylate solutions could not explain the results obtained.

Analysis of fractions

As the work on extraction had shown that definite individual fractions of the gluten protein were difficult to isolate by solubility methods, it was not surprising to find that the results of amide analyses of these fractions failed to show the variation exhibited by the fractions obtained by precipitation. There were many factors that combined to render these results unreliable, however, so other methods of obtaining fractions for chemical analysis were sought.

The extraction of individual samples of gluten by various concentrations of sodium salicylate had yielded results showing systematic variation in the gluten from the most to the least soluble fractions. By selecting a suitable concentration of salicylate, the gluten could be separated into dispersed and non-dispersed portions at any desired point. These dispersed and non-dispersed portions appeared to be the best material for use in amide analyses, since any differences in amide content of the various portions must be the result of differences in amide content of the fractions removed by progressively more concentrated salicylate. The "fractions" thus obtained could not be formed by the type of adsorption discussed by Blish, as no fractional precipitation was employed.

Eight gluten balls were prepared from 10-gm. flour samples. Two balls were broken up and extracted four times with 2%, two balls with 3%, two with 5%, and two with 8% sodium salicylate solutions. The protein not dispersed was hydrolyzed, and amide nitrogen determined. The dispersions in sodium salicylate were made up to one-fifth saturation with magnesium sulphate and all the precipitated material was washed, hydrolyzed and analyzed. Earlier work had shown that one-fifth saturation with magnesium sulphate precipitated the protein fraction that was highest in amide nitrogen content. The fraction not precipitated by this concentration, but precipitated by one-half saturation, was much lower in amide (12). The lower salt concentration was used, therefore, because it differentiated best between the fractions considered to belong to the true gluten complex and those considered as distinct.

It was necessary, however, to determine whether the protein that was considered as not belonging to the gluten complex was as distinct when isolated by solubility methods as it was when isolated by precipitation. For this reason, duplicate gluten balls were extracted with 1% sodium salicylate, which removes only the more soluble portion of the gluten protein, and the extract was concentrated and hydrolyzed. Amide nitrogen was determined on the hydrolysate.

The amide results for the two experiments, plotted against the midpoints of the gluten fractions, are presented in Fig. 3. Results obtained with a

similar flour by use of fractional precipitation methods (12) are also included in Fig. 3.

These results show that the distribution of amide nitrogen in successive gluten protein fractions is similar, whether the fractionation is carried out by solubility or precipitation methods. The latter method results in a sharper

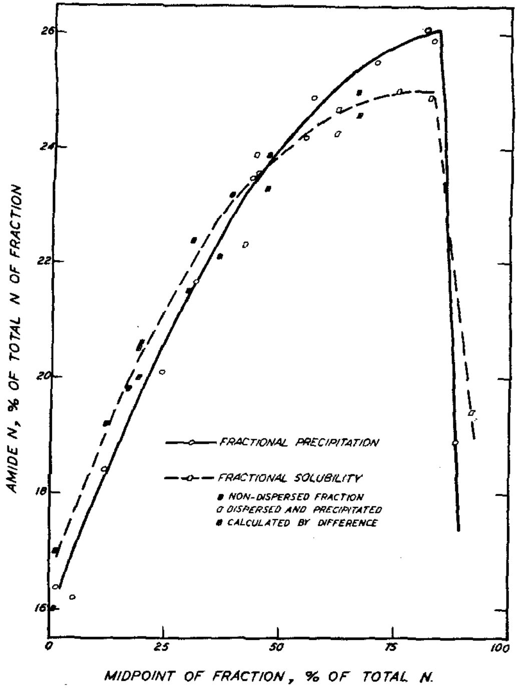

FIG. 3. *Amide nitrogen in gluten fractions obtained by fractional solubility and by fractional precipitation methods.*

separation of specific fractions, however, since there is no question of contamination of successive fractions by small amounts of unextracted portions of more soluble protein. This is shown by the fact that all but one of the points that were obtained by analysis of non-dispersed protein fall above the

general curve obtained by precipitation (Fig. 3), while all but one of those obtained by analysis of dispersed protein fall below it.

As a result of somewhat incomplete extraction, the non-dispersed protein contains small amounts of the more soluble fractions, which are, for the most part, higher in amide than the less soluble protein. This tends to raise the amide content of the non-dispersed fractions. Similarly, in precipitating the protein dispersed in salicylate, small amounts of the most soluble fractions, which are low in amide, are carried down with the precipitate. This tends to lower the amide content of the dispersed fractions, the effect being most marked with the smallest fractions. While each fraction obtained by total precipitation of the protein dispersed in various concentrations of salicylate shows this effect, only one fraction obtained by fractional precipitation can show it, because by the latter method the fractions are obtained successively from the same dispersion, and not separately from different dispersions. Thus the results obtained with fractional precipitation methods do not show a depression in amide content of the more soluble portions.

The results obtained with the concentrated 1% extract agree very well with those for the final precipitated fractions. Extraction was almost certainly incomplete with 1% salicylate, placing the midpoint of the fraction too far to the right on the graph. Had extraction been complete, the sharp breaks in the curves would have agreed even better than they do.

It has already been stated that the differences between the amide content of the various portions must be the result of differences in amide content of the fractions removed by progressively more concentrated solutions. The amide content of such extracts can be calculated from the weights of total and amide nitrogen. Furthermore, the calculations can be duplicated because the fraction in question appears once in the non-dispersed, and once in the dispersed, portion. The results for one experiment together with corresponding midpoints are presented in Table IV. These results have also been plotted in Fig. 3, and substantiate in every way the conclusions drawn from the direct analytical results.

TABLE IV

AMIDE CONTENT OF SPECIFIC FRACTIONS, OBTAINED BY CALCULATION OF DIFFERENCES IN SUCCESSIVE FRACTIONS

Concentrations of sodium salicylate between which solubility of fraction lies	Midpoint of fraction, % of total nitrogen	Amide nitrogen content	
		Non-dispersed	Dispersed
8–5	19.0	20.0	20.5
5–3	47.4	23.9	23.3
3–2	66.2	24.6	25.0

Discussion

It is now possible to consider the findings and conclusions of Blish as a result of fractional solubility studies. Two principal experiments are involved.

In the first experiment, Blish reports that more than 30% of the flour protein was extracted with water and that successive "fractions" of this extract did not vary in chemical composition. This was taken as definitely contradicting the evidence afforded by fractional precipitation data of the type presented by McCalla and Rose.

It was pointed out in the original paper that approximately 20 to 30% of the flour protein did not belong to the true gluten protein complex. Actually, 17.4% of the total nitrogen of one of the flours was "extracted" with water during gluten washing, and 16.1% more was extracted from the gluten with distilled water. Thus 33.5% of the flour nitrogen did not belong to the gluten complex. It is now apparent that the 16.1% extracted from this gluten was too low, as only a single extraction was made. That the successive "fractions" of the water-extracted protein did not vary in composition is to be expected, as these are not fractions, in the sense that we use the term, but are rather portions of the same fraction. In all our work it has been found that successive portions of the protein extracted with one solvent possess the same chemical composition. Thus we can agree with Blish's results, but not with his interpretation. Furthermore, his results were obtained with protein that did not belong to the true gluten complex, and therefore could not be accepted as definite contradiction of results or conclusions based on this complex, even if he had been dealing with distinct fractions.

In the second experiment (2), a single sample of flour was progressively extracted with sodium salicylate solutions of increasing concentration. A comparison of the chemical composition of the fractions revealed no evidence of progressive and systematic variations in chemical properties. The data were not presented, but it was concluded that they supported the idea that there are few components or component groups. As already described in a previous section, a similar experiment was carried out during the present studies. In this work single extractions of gluten with the lower concentrations of salicylate failed to remove more than small portions of the fractions specifically soluble in these concentrations, with the result that the remainder was carried over to successive extracts. The final result of this was a marked flattening of the amide curve, and a loss of individuality of fractions. Repeated extraction with a single concentration of salicylate, followed by repeated extraction with the next higher concentration, brought the results closer to those obtained by other methods, but in this case denaturation of the protein was so apparent that there was undoubtedly overlapping in the solubility of fractions that were originally distinct. We should hesitate to draw conclusions in support of the earlier hypothesis from these data, but we cannot accept the results as indicative of the true nature of gluten. On the basis of the results presented in the preceding section, the failure of these to conform to those previously presented can be reasonably explained; and the technical difficulties met in studying such fractions engenders little confidence in the results.

As a result of the present study it must be concluded that the technique involved in fractional solubility studies is much less satisfactory than that involved in precipitation. As successive extractions with low concentrations of salicylate not only increase the amounts of protein removed, but also denature the non-dispersed portions, complete extraction of any specific fraction is rendered difficult, and the complete removal of a second fraction is apparently impossible by our present methods. This is not so when dealing with relatively concentrated solutions, because penetration is rapid and complete. In this range, the results with individual fractions obtained by solubility methods agree with those obtained using precipitation methods.

As Blish points out, two interpretations of the results are possible when there is a systematic variation in physical and chemical properties of successive fractions. In the previous publication (12), these were interpreted as indicating an indefinite number of components in a single protein complex. Blish's viewpoint, however, is that there are relatively few main fractions, although the question as to the similarity of components within fractions is left open. The first view implies many distinct fractions, the second, mixtures containing progressively varying proportions of a few fractions. The present work on fractional solubility does not permit of final and dogmatic conclusions, but the results are in agreement with those obtained by fractional precipitation. The fractions could not have been the result of adsorption of one main component on another, as dispersion of a specific fraction only is effected. The possibility of the complex formation described by de Jong (6) influencing the formation of the fractions isolated cannot be dismissed; but preliminary results of an investigation designed to test this suggestion indicate that fractions occurring at any specific point in the gluten complex are of the same chemical nature, regardless of the pH of the salicylate solution. Further discussion of this phase of the work is unwarranted at the present time.

The possibility that the fractions might be formed as a result of overlapping solubilities of a few components implies that these main components must vary in solubility within themselves. If such variation is sufficient to produce fractions such as were isolated in the present study, we are probably justified in considering the different portions of the components as individual fractions.

It is perhaps well to repeat Blish's warning that a new discovery in this field may invalidate many of the conclusions reached as a result of previous studies. A final statement as to the composition of the gluten protein is impossible at the present time, but until more exact separation of the fractions is accomplished, we prefer to maintain the position outlined in the previous paper (12), and consider the main gluten protein as a single complex that can be divided into many fractions differing systematically in both physical and chemical properties.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

IV. COMMONLY MEASURED PROPERTIES AND THEIR CORRELATIONS WITH NITROGEN AND 1000-KERNEL WEIGHT[1]

By W. O. S. Meredith[2] and J. Ansel Anderson[3]

Abstract

Samples representing 12 varieties of barley grown at 12 widely separated experimental stations in Canada were malted and subsequently analyzed. Varietal differences were found in steeping rate, malting loss, sprouts, extract, wort nitrogen and diastatic activity.

O.A.C. 21 and Mensury, varieties which Canadian maltsters prefer, gave high values for all six properties. Olli, which maltsters consider promising, gave still higher values. Pontiac equalled O.A.C. 21 only in diastatic activity, and the remaining six-rowed, rough-awned variety, Peatland, which has proved less satisfactory, gave lower values for all properties, and this inferiority was more apparent when adjustments were made for its high nitrogen content. In general, the six-rowed, smooth-awned varieties, particularly Regal and Wisconsin 38, gave much lower values. However, Nobarb was only 1% lower in extract than O.A.C. 21, and Velvet equalled the latter in wort nitrogen and diastatic activity. The two-rowed varieties, Charlottetown 80, Hannchen and Victory, were higher in extract but lower in other malt properties than O.A.C. 21. Hannchen, of which considerable quantities are malted in the United States, proved most similar to O.A.C. 21.

Amongst the *inter-varietal* correlations between malt properties and nitrogen content or 1000-kernel weight of the barley, only the partial correlations for diastatic activity and 1000-kernel weight, independent of nitrogen, proved significant ($r = 0.609$). Amongst the *inter-station* correlations, indicative of *intra-varietal* associations, those for nitrogen and extract ($r = -0.957$), nitrogen and diastatic activity ($r = 0.962$), and nitrogen and wort nitrogen ($r = 0.764$), surpassed the 1% level of significance; whereas those for nitrogen and steeping time ($r = -0.637$), nitrogen and malting loss ($r = 0.694$), and 1000-kernel weight and steeping time ($r = 0.652$), surpassed the 5% level. The inter-station multiple correlation coefficient for steeping time and nitrogen and 1000-kernel weight ($R = 0.840$) proved highly significant. The corresponding multiple correlation coefficient for extract was not significantly higher than the coefficient of correlation with nitrogen alone

The investigations described in this series of papers were undertaken with the object of collecting an adequate body of data for the statistical examination of the correlations between various barley and malt properties, and of studying the bearing of these on the problem of evaluating the malting quality of new varieties of barley. For this purpose 144 samples of barley and the malts made from them are being analyzed. The samples represent 12 varieties of barley grown at each of 12 widely separated experimental stations in Canada.

The first three papers of this series dealt with the total nitrogen and nitrogen fractions of the barley (2), the saccharifying activities of the barley and malt (14), and the correlations between these properties (7). The present paper describes the methods used in making the malts, and deals mainly with the

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[2] *Senior Research Assistant, Associate Committee on Grain Research, Department of Plant Science, University of Manitoba, Winnipeg.*
[3] *Biochemist, Division of Biology and Agriculture, National Research Laboratories, Ottawa.*

rate of steeping, malting loss, extract, wort nitrogen and diastatic activity, and their correlations with total nitrogen content and 1000-kernel weight of the barley. These properties and a number of the relations have been studied by previous investigators (8–12, 14, and others), so that this paper presents few new generalizations. It is published mainly with the object of presenting data that provide additional information on the 12 varieties we are studying, and to which we shall also have occasion to refer in other papers of this series.

Materials and Methods

Materials

The barley samples used in the present investigation were described in detail in Part I of this series of papers (2). Briefly, they consist of 144 samples representing 12 varieties grown at each of 12 widely separated experimental stations in Canada. The varieties are listed in Table I and the stations in Table III.

Malting Method

In order to control systematic errors that might arise in malting a series of samples which required three months to complete, the malting was carried out in the following manner. Each batch of malt contained 12 samples representing the 12 varieties grown at one station. The samples were arranged in random order within batches and the batches were malted in random order. The first replicates were made in the first 12 batches and the second replicates in the succeeding 12 batches. It will be apparent that these methods provide for an unbiased comparison of the varieties.

Before malting, the thin barley and screenings were removed from each sample by passing it through a ring grader, with rings 5/64 in. apart, driven at constant speed by means of an electric motor and a reducing gear. The amount of plump barley recovered is reported as a percentage of the original sample weight.

The cleaned barley was then divided into two sub-samples by means of a Boerner sampler, and an aliquot part of each sub-sample, representing 25 gm. of barley dry matter, was used to determine the steeping time required to bring the moisture content of the barley to 46%. A larger aliquot part of each sub-sample, representing 250 gm. of barley dry matter, was subsequently malted in the laboratory equipment described by Anderson and Rowland (4).

The samples were steeped at 50° F. to a moisture content of 46%. They were then placed in the germination chamber, which was operated at an air temperature of 53° F. After 64 hr. each sample was watered to a moisture content of 48%, the amount of water required varying between 15 and 25 gm. After 144 hr. the samples were transferred to the kiln and dried under the following conditions: 0 to 6 hr., temperature rising at constant rate from 85° to 120° F.; 6 to 22 hr. at 120° F.; 22 to 30 hr., temperature rising at constant rate from 120° to 150° F.; 30 to 40 hr. at 150° F.; 40 to 42 hr., temperature rising at constant rate from 150° to 175° F.; 42 to 46 hr. at 175° F.

Malting loss and percentage sprouts (roots) were determined immediately after kilning. The sample was kneaded for one minute in a small bag. The roots that were rubbed off by this process were removed by sifting, and roots and finished malt were weighed. The percentage malting loss was calculated from data on the weights and moisture contents of the barley sample and the finished malt. The figures reported for sprouts are rather low, since some of these are rubbed off and lost during the kilning process.

The authors also wish to point out that the malts were somewhat under-modified. As a result it seems probable that maximum extract yields and complete development of enzymatic activity were not obtained. However, as previous investigations (3, and earlier papers in that series) have shown that the comparative order of varieties is little affected by moderate changes in malting conditions, we believe that the comparative results of the present investigation were not materially affected by the fact that the malts were not fully modified.

Analytical Methods

Determinations of the nitrogen content and 1000-kernel weight of the barley were made on cleaned samples. The former was determined by a Kjeldahl method on duplicate samples of barley ground separately. The latter was determined by weighing out duplicate 25-gm. samples and counting the number of kernels in each.

The following properties were determined by the Official Method of the American Society of Brewing Chemists (1): malt extract and moisture; color, odor, clarity, and speed of filtration of the wort; and saccharification time. Diastatic activity was determined by a ferricyanide modification of the Official Method (5). Wort nitrogen was determined on a 25-ml. aliquot of the laboratory wort by adding a few drops of acid, evaporating to a thin syrup, and making a Kjeldahl determination. It is reported as a percentage of the malt dry matter.

Results and Discussion

Varietal Differences

The results of the investigation are summarized in Table I as means, over all stations, for each variety. Owing to the differential effect of environment on varieties, these did not all fall in the same order with respect to any property at all stations. It was therefore necessary to resort to statistical analyses in order to determine whether the differences between varietal means could be considered significant. The results of the statistical analyses are given in a later section (Table VI), but are summarized in the last line of Table I as necessary differences between means required for a 5% level of significance, *i.e.*, for odds of 19 to 1 that a real difference between varieties is operating to spread the means.

A comparison of the necessary differences with the actual differences between the means for the individual varieties leaves no room for doubt that varietal differences exist with respect to each property measured. The results of the

TABLE I

BARLEY, MALTING, AND MALT PROPERTIES, MEANS FOR EACH VARIETY

Class	Variety	Barley			Malting data			Malt		
		Total nitrogen, %	1000-kernel wt., gm.	Plump barley, %	Steeping time, hr.	Malting loss, %	Sprouts, %	Extract, %	Wort nitrogen, %	Diastatic power, °L.
Six-rowed, rough-awned	O.A.C. 21	2.242	31.0	74.3	75	7.4	2.2	73.9	0.82	127
	Mensury, Ott. 60	2.303	32.1	77.1	78	7.3	2.1	73.9	0.85	129
	Olli	2.215	29.6	63.5	55	7.7	2.1	76.0	0.93	153
	Peatland	2.471	28.6	66.7	80	7.1	1.8	73.2	0.80	120
	Pontiac	2.278	32.5	79.3	78	6.9	1.9	72.5	0.76	131
Six-rowed, smooth-awned	Nobarb	2.194	33.8	80.2	82	6.4	1.6	72.9	0.66	100
	Regal	2.352	31.6	76.7	86	7.0	1.7	71.1	0.74	85
	Velvet	2.372	31.1	76.8	80	6.6	1.7	72.3	0.80	124
	Wisconsin 38	2.240	32.4	86.1	91	6.6	1.6	71.2	0.64	95
Two-rowed, rough-awned	Charlottetown 80	2.298	35.1	82.5	78	7.6	2.1	76.1	0.76	100
	Hannchen	2.215	35.6	83.9	79	7.2	1.9	76.9	0.80	116
	Victory	2.228	36.1	83.9	78	7.1	2.1	75.9	0.71	103
Mean over all varieties		2.284	32.4	77.0	78	7.1	1.9	73.8	0.77	115
Necessary difference, 5% level		0.079	1.5	8.5	5	0.4	0.3	0.7	0.04	11

investigation are thus in agreement with those of other investigators who have studied these properties.

Canadian maltsters have found by experience that O.A.C. 21 and Mensury Ott. 60 are very satisfactory for the production of brewers' malt for domestic consumption. These varieties grow readily during the malting process, as is shown by data for malting loss and sprouts, and produce malts characterized by fairly high extract yields, high diastatic activity, and high percentages of wort nitrogen.

As a result of many laboratory tests and some commercial cage malting tests, the maltsters are inclined to consider Olli a very promising variety, and commercial scale tests will be made with it in the near future. The variety is a comparatively new introduction which matures very early and is suited only to northern districts. It gives an extremely high extract yield for a six-rowed variety and is characterized by high enzymatic activity as is shown by the data for diastatic power, wort nitrogen, malting loss and sprouts. It tends to produce grain of low nitrogen content but has rather small kernels.

Peatland has been malted commercially and was at one time considered a promising variety. It is now considered of doubtful value for malting purposes, largely, we believe, because of its tendency to produce grain of very high nitrogen content. In other respects it appears to have much the same properties as O.A.C. 21.

Information on the malting quality of Pontiac is confined to the results of laboratory tests. The variety does not seem promising since it grows slowly and the malt is rather low in extract yield and percentage of wort nitrogen.

Canadian maltsters do not consider any of the four smooth-awned varieties listed suitable for malting purposes. On the other hand large quantities of Velvet and Wisconsin 38 are malted in the United States, and of these two varieties the former is considered superior (11). Velvet appears to grow rather more slowly than O.A.C. 21. It also yields lower extract values, but is equal to O.A.C. 21 with respect to diastatic activity and wort nitrogen. Nobarb gives a higher extract than Velvet, but it and the other two smooth-awned varieties give lower values for the other main malt properties.

The two-rowed varieties are characterized by high 1000-kernel weight and high extract yield. Since the amounts of two-rowed barley malted in Canada are extremely small, information on the comparative malting qualities of these three varieties is confined largely to the results of the present investigation. It is interesting to note, however, that Hannchen, of which considerable quantities are malted in the United States, is higher in diastatic activity and percentage wort nitrogen than the other two varieties.

No varietal differences were demonstrated with respect to certain minor properties of the malt and wort. In these circumstances it seems necessary only to list the properties in question, together with average values for them: malt moisture, 3.3%; color of wort, 1.6 Lovibond units; odor of wort, aromatic; appearance of wort, clear; speed of filtration, normal; and time of conversion, less than 5 min.

Varietal Differences at Equal Nitrogen Contents

The interpretation of the data on malt properties is complicated by the existence of varietal differences in total nitrogen content and of intra-varietal relations between nitrogen and the malt properties. In these circumstances it seems useful to compare the varieties at equal nitrogen contents. This can be effected by determining the coefficients of regression of each malt property on total nitrogen and using these to adjust the varietal means for each property to the values corresponding to a total nitrogen content of 2.28%, which figure represents the mean nitrogen content of all varieties.

It is apparent that this procedure will be useful only if the regression coefficients for the individual varieties are almost identical, since if they are not, the comparative order or relative positions of the varieties would depend upon the nitrogen content at which the comparison of adjusted means was made. Statistical analyses, reported in a later section (Table VII), showed that the varietal regression coefficients for extract on total nitrogen did not differ significantly, nor did those for wort nitrogen on total nitrogen. The adjusted means for these properties were accordingly calculated and are reported in Table II. For the information of those who consider an index of nitrogen modification useful, wort nitrogen is also reported as a percentage of total nitrogen of the barley. In this connection it should be noted that total wort nitrogen rather than the more commonly determined permanently soluble nitrogen was used in calculating the indices of nitrogen modification.

TABLE II

VARIETAL MEANS FOR MALT PROPERTIES ADJUSTED BY CALCULATION TO VALUES CORRESPONDING TO TOTAL NITROGEN CONTENT OF BARLEY OF 2.28%

Class	Variety	Extract, %	Wort nitrogen		Diastatic activity, °L.	
			Per cent of dry matter	Per cent of total nitrogen	At total nitrogen of 2.28%	At total nitrogen of 1.80%
Six-rowed, rough-awned	O.A.C. 21	73.7	0.83	36.4	128	93
	Mensury, Ott. 60	74.0	0.84	36.8	128	90
	Olli	75.7	0.94	41.2	158	122
	Pentland	74.1	0.76	33.3	105	66
	Pontiac	72.5	0.76	33.3	131	92
Six-rowed, smooth-awned	Nobarb	72.5	0.68	29.8	105	79
	Regal	71.5	0.73	32.0	82	60
	Velvet	72.7	0.77	33.8	116	75
	Wisconsin 38	71.0	0.65	28.5	97	78
Two-rowed, rough-awned	Charlottetown 80	76.0	0.75	32.9	100	87
	Hannchen	76.6	0.81	35.5	121	88
	Victory	75.7	0.72	31.6	105	86
Mean, over all varieties		73.8	0.77	33.8	115	85
Necessary difference, 5% level		0.5	0.03	1.3	11	8

Statistical analyses showed that the varietal regression coefficients for diastatic activity on nitrogen content differed significantly. (This point is illustrated by the scatter diagrams given in Fig. 2 of Part III of this series.) Accordingly, adjustment of the mean diastatic activities to a given nitrogen content presents an incomplete picture of varietal differences. In order to overcome this difficulty, adjusted means were calculated not only for a nitrogen content of 2.28% but also for 1.80%. These values probably represent about the lower and upper limits of the nitrogen content of barley malted commercially in Canada. The two sets of adjusted varietal means for diastatic activity are given in the last two columns of Table II.

The effect of adjusting for differences in total nitrogen content is most marked with respect to Peatland. After adjustment this variety has as high an extract yield as O.A.C. 21 but gives lower values for both wort nitrogen and diastatic activity. It thus appears that when comparisons are made at equal nitrogen contents, Peatland is low in enzymatic activity, which may well be an additional reason for its failure to find favor with the maltsters.

The data for total wort nitrogen as percentage of total barley nitrogen form a so-called index of modification. Since all varieties were malted under the same conditions it is apparent that O.A.C. 21, Mensury, Hannchen, and particularly Olli, modify much more readily than the other varieties, and these are the four varieties that seem most satisfactory to the maltsters. It may also be argued that the lower values given by other varieties indicate that these were not malted advantageously, and that their indices of modification could be raised by changing the malting conditions. Previous investigations (3, and earlier papers in that series) suggest that there is some truth in this hypothesis. We believe, however, that varietal differences in indices of modification would persist even if each variety were malted under optimum conditions. This is the opinion held by Thunaeus and Schröderheim (15) as a result of their investigations of Swedish barleys.

The data for diastatic activity show that, owing to differences in the varietal regression coefficients, the comparative orders of the varieties are not identical when adjustments are made to correspond with different nitrogen levels. It will be noted, however, that at both levels Olli gives by far the highest values, and is followed by a group of four varieties, O.A.C. 21, Mensury, Pontiac and Hannchen. At both nitrogen levels Regal also yields the lowest values for diastatic activity.

Station Differences

The results of the investigation are summarized in Table III as means, over all varieties, for each station. The stations are listed in order of increasing nitrogen content of the barley in order to facilitate examination of the relations between nitrogen and other properties, a subject that is discussed in the next section.

The data show that environmental differences among the stations resulted in the production of a series of samples covering a wide range of values with

TABLE III

BARLEY, MALTING, AND MALT PROPERTIES, MEANS FOR EACH STATION

Station	Barley			Malting data			Malt		
	Total nitrogen, %	1000-kernel weight, gm.	Plump barley, %	Steeping time, hr.	Malting loss, %	Sprouts, %	Extract, %	Wort nitrogen, %	Diastatic power, °L.
Nappan	1.540	34.8	92.2	99	6.8	1.6	78.0	0.61	63
Fredericton	1.738	34.8	84.7	83	6.5	1.7	76.3	0.65	85
Ste. Anne de Bellevue	1.932	32.9	79.3	74	7.0	1.6	75.5	0.78	100
Ste. Anne de la Pocatière	2.278	33.5	89.5	77	6.9	2.0	74.3	0.82	121
Lethbridge	2.294	33.5	86.6	84	7.2	2.0	74.7	0.73	116
Winnipeg	2.333	30.6	65.0	75	7.2	2.3	73.0	0.75	105
Brandon	2.357	28.0	84.0	77	7.0	2.0	73.9	0.78	117
Guelph	2.381	32.5	50.3	65	6.6	1.8	72.8	0.87	122
Ottawa	2.526	28.0	69.7	81	7.4	1.9	73.3	0.87	133
Lacombe	2.668	32.5	82.9	75	7.1	2.0	71.9	0.79	140
Beaver Lodge	2.674	31.8	78.0	74	8.0	2.4	70.5	0.78	149
Gilbert Plains	2.687	30.8	67.3	76	7.4	1.7	71.6	0.81	133
Mean over all stations	2.284	32.4	77.0	78	7.1	1.9	73.8	0.77	115
Necessary difference, 5% level	0.079	1.5	1.5	5	0.4	0.3	0.7	0.04	11

respect to each of the properties studied. The spread in malting quality is wider than that between the best and poorest samples malted commercially in Canada. However, the stations were selected with the object of obtaining wide differences in malting quality, in order to facilitate the demonstration of such relations between the various barley and malt properties as may exist.

Correlations between Total Nitrogen and Other Properties

The correlations between total nitrogen and other properties were examined by calculating the correlation coefficients for varietal means and station means. The resulting statistics are given in Table IV. None of the coefficients for varietal means attained the 5% level of significance, so that the investigation yields no evidence of inter-varietal relations between nitrogen and other properties.

TABLE IV
INTER-VARIETAL AND INTER-STATION CORRELATIONS FOR NITROGEN CONTENT AND OTHER PROPERTIES

Property	Correlation coefficient	
	Inter-varietal	Inter-station
1000-kernel weight	−.552	−.519
Plump barley	−.338	−.440
Steeping time	.238	−.637*
Malting loss	−.041	.694*
Sprouts	−.061	.640*
Extract	−.380	−.957**
Diastatic activity	−.039	.962**
Wort nitrogen as % of dry matter	.166	.764**

NOTE: *In this and later tables ** denotes that the 1% level, and * that the 5% level of significance is attained.*

Reference to Table III, which gives the mean values for each station, will show that as the total nitrogen increases, malt extract tends to decrease, and malting loss, sprouts, wort nitrogen and diastatic activity tend to increase. The degree of association between nitrogen and these other properties is summarized by the correlation coefficients between station means given in the last column of Table IV. It is quite close for extract and diastatic activity, but very considerably lower for the other properties. All the associations, except that between nitrogen and steeping time, have been discussed by earlier investigators and further comment on them seems unnecessary.

Correlations between 1000-kernel Weight and Other Properties

The inter-varietal and inter-station coefficients of correlation between 1000-kernel weight and other properties are given in the first two columns of data in Table V. Owing to the association between 1000-kernel weight and nitrogen it appeared that these statistics might be misleading, since they might merely, or at least partially, reflect correlations with nitrogen, rather than represent actual correlations with 1000-kernel weight. The possible effects of nitrogen were therefore eliminated by calculating the partial correlation coefficients for 1000-kernel weight and each property, independent of nitrogen. These are listed in the last two columns of Table V.

A comparison of the simple and partial coefficients shows that in some instances the former did partially reflect correlations with nitrogen, whereas

in other instances the association with nitrogen tended to mask the true association with 1000-kernel weight. Only the partial correlation coefficients appear to merit further discussion.

TABLE V

INTER-VARIETAL AND INTER-STATION CORRELATIONS FOR 1000-KERNEL WEIGHT AND OTHER PROPERTIES, AND PARTIAL CORRELATIONS INDEPENDENT OF NITROGEN CONTENT

Property	Correlation coefficient		Partial correlation coefficient	
	Inter-varietal	Inter-station	Inter-varietal	Inter-station
Total nitrogen	−.552	−.519	—	—
Plump barley	.361	.933**	.191	.840**
Steeping time	.289	.756**	.525	.652*
Malting loss	−.099	−.173	−.146	.304
Sprouts	.120	−.200	.104	.202
Extract	.439	.600	.297	.416
Diastatic activity	−.486	−.434	−.609*	.276
Wort nitrogen as % of dry matter	−.490	−.654*	−.485	−.466

Amongst the inter-varietal partial correlations only that for diastatic activity is significant, but the association between the two variables is by no means close. We may assume, however, that there is some tendency for varieties that are lower in 1000-kernel weight to be higher in diastatic activity.

The inter-station partial correlation coefficients show that there is a fairly close intra-varietal relation between percentage plump barley and 1000-kernel weight of the plump barley. This was to be expected and it seems peculiar that no such inter-varietal relation exists.

It is also apparent that there is an inter-station association between 1000-kernel weight and steeping time, which indicates that within any variety samples of smaller 1000-kernel weight tend to absorb water more rapidly. Although the corresponding inter-varietal correlation coefficient does not attain the 5% level of significance, it is not far below this, and thus suggests that a similar inter-varietal association may well exist. On common sense grounds we should expect both an inter- and an intra-varietal relation between 1000-kernel weight, which is a measure of kernel size, and rate of water absorption. The surprising thing is that the degree of association between these two variables is not greater. It is thus apparent that although 1000-kernel weight is one of the factors controlling rate of water absorption, there must be other factors. Data presented in the next section show that some of these factors are associated with the nitrogen content of the grain.

Multiple Correlations between Certain Properties, Nitrogen and 1000-kernel Weight.

Steeping time. The simultaneous association between steeping time, nitrogen and 1000-kernel weight was investigated by calculating multiple correla-

tion coefficients representing the correlation between steeping time as determined and steeping time as predicted from nitrogen and 1000-kernel weight. The data were also analyzed in order to determine whether the prediction based on two independent variables could be considered significantly better than prediction based on one alone (see Table IX). This proved true for the inter-station multiple coefficient (R = 0.826), which surpassed the 1% level of significance. It thus appears that within each variety the time required for the absorption of 46% of moisture depends not only on the kernel weight but also on some other factor, or factors, associated with nitrogen content. However, since the multiple correlation coefficient is not very high it also follows that some factor, or factors, associated with neither nitrogen nor 1000-kernel weight, also play a part in controlling rate of water absorption.

The inter-varietal multiple correlation coefficient (R = 0.546) fails to attain the 5% level of significance. Moreover, it is little higher than the partial correlation coefficient for steeping time and 1000-kernel weight, independent of nitrogen (r = 0.525). It is thus apparent that between varieties, factors closely associated with nitrogen content have little influence on steeping time. It appears, however, that 1000-kernel weight has some influence although it is not a factor of major importance.

Extract. As Bishop and Day (10, and earlier papers) have made use of both nitrogen and 1000-kernel weight for predicting extract, it seemed advisable to determine whether our data would show a significant increase in the precision of prediction through the introduction of 1000-kernel weight as a second independent variable. The inter-station multiple correlation coefficient (R = 0.964) proved to be slightly, but not significantly, higher (see Table IX) than the coefficient of correlation between extract and nitrogen alone (r = −0.957). It thus appears that the advantage to be gained by the introduction of the second independent variable is very small.

The inter-varietal multiple correlation coefficient failed to attain, or even to approach closely, the 5% level. There are thus no grounds for believing that varieties that are low in nitrogen and high in 1000-kernel weight will tend to be higher in extract yield than varieties having the opposite characteristics.

Diastatic activity. The simultaneous association between diastatic activity, nitrogen, and 1000-kernel weight, was discussed in Part III (7).

Other Relations

The data presented in this and preceding papers (2, 7, 14) make possible the investigation of a number of other correlations between barley and malt properties. A study of these is being undertaken and the results will be presented in further papers of this series.

Statistical Analyses

The methods used for analyzing the data reported in this paper are the same as those used under similar circumstances for analyzing data given in Parts I and III. In these circumstances it seems unnecessary to discuss the statistics in detail, although it seems advisable to present them in order to substantiate statements made previously in this paper.

The variance of the data for each property was analyzed separately and the resulting mean squares are given in Table VI. Before varietal means were adjusted for differences in total nitrogen (see Table II), the homogeneity of varietal regression coefficients was tested by means of analyses of residual inter-station, intra-varietal variance. The results of these tests are given in Table VII. Only the varietal regressions for diastatic activity on nitrogen proved unhomogeneous. Varietal means for extract and wort nitrogen were adjusted for differences in total nitrogen and the significance of the differences between the adjusted means was tested in the usual way by calculating the adjusted residual mean squares. These are given in Table VIII. Analyses of var-

TABLE VI

ANALYSES OF VARIANCE FOR BARLEY, MALTING AND MALT PROPERTIES: MEAN SQUARES

Property	Variance due to		
	Stations	Varieties	Interaction
Total nitrogen, %	1.651**	0.079**	0.009
1000-kernel weight, gm.	44.71**	65.97**	3.55
Plump barley, %	1769**	549**	108
Steeping time, hr.	801.2**	905.6**	41.9
Malting loss, %	1.775**	1.977**	0.265
Sprouts, %	0.789**	0.608**	0.095
Extract, %	53.57**	46.56**	0.60
Diastatic power, °L.	7052**	4372**	166
Wort nitrogen as % of dry matter	0.0725**	0.0770**	0.0018
Degrees of freedom	11	11	121

TABLE VII

TEST OF HOMOGENEITY OF VARIETAL REGRESSION COEFFICIENTS BY ANALYSIS OF RESIDUAL VARIANCE

Variance due to	Degrees of freedom	Mean squares		
		Extract	Wort nitrogen	Diastatic activity
Differences among varietal regression coefficients	11	0.333	0.0025	686.5**
Deviations from individual varietal regressions	*120*	*0.808*	*0.0039*	119.0

TABLE VIII

TEST OF SIGNIFICANCE OF VARIANCE IN EXTRACT AND WORT NITROGEN ADJUSTED FOR DIFFERENCES IN TOTAL NITROGEN

Variance due to	Degrees of freedom	Residual mean squares	
		Extract	Wort nitrogen
Varieties	11	40.816**	0.07515**
Stations	11	4.630**	0.03078**
Remainder	120	0.415	0.00131

iance were also used to determine whether the prediction of steeping time, extract, and diastatic activity, from barley nitrogen could be improved by the introduction of 1000-kernel weight as a second independent variable. The resulting statistics are given in Table IX.

TABLE IX

TEST OF SIGNIFICANCE OF DIFFERENCE BETWEEN SIMPLE COEFFICIENT OF CORRELATION WITH NITROGEN AND MULTIPLE COEFFICIENT OF CORRELATION WITH NITROGEN AND 1000-KERNEL WT.

Dependent variable	Variance accounted for by	Degrees of freedom	Mean square	
			Varieties	Stations
Steeping time	Total nitrogen	1	564	3576**
	Added effect of 1000-kernel wt.	1	2406	2169*
	Residual	9	777	341
Extract	Total nitrogen	1	74.14	539.29**
	Added effect of 1000-kernel wt.	1	38.76	8.66
	Residual	9	44.36	4.59
Diastatic activity	Total nitrogen	1	73	71714**
	Added effect of 1000-kernel wt.	1	17831*	445
	Residual	9	3355	602

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EFFECT OF PHYTOHORMONE DUSTS ON GROWTH AND YIELD OF WINTER WHEAT VARIETIES[1]

By G. P. McRostie[2], J. W. Hopkins[3] and N. H. Grace[4]

Abstract

Various concentrations of indolyl- and naphthylacetic acids were applied to ten varieties of winter wheat prior to planting in a replicated field trial, one-half of each plot receiving seed dusted with Ceresan plus phytohormone, and the other half receiving an equal quantity of seed of the same variety dusted with Ceresan only.

Some differences between the hormone-treated and untreated sub-plots in respect of early growth and subsequent density of stand were apparent to visual inspection. There were also statistically significant differences in respect of straw production, and of yield, weight per bushel, and nitrogen content of grain. The effects on grain yield were complicated by the differential response of varieties to the same treatment, and at the higher concentrations some depressions of yield resulted. On the average both chemicals tended slightly to reduce the nitrogen content of the grain produced.

The results as a whole seem to demonstrate the physiological activity of both substances tested when applied in this way. Further investigation of dosages, varietal characteristics, and seasonal effects, however, will be required before general conclusions can be reached.

Preliminary tests, by one of the authors, of the physiological activity of certain phytohormone chemicals when applied in the form of dusts to seeds prior to planting, have already been reported (1). In view of the promising indications obtained, it was desired to ascertain whether these substances had any appreciable effect on the growth and yield of crop plants under field conditions.

As winter wheat might perhaps be expected to respond particularly advantageously to any induced stimulation of germinative or early vegetative vigor, a replicated varietal trial of this crop, with and without phytohormone dust treatment, was laid down at the Ontario Agricultural College, Guelph, in the autumn of 1937. The present communication describes the results secured from this trial in the season 1937–38.

Experimental

The hormone trial was superimposed upon the annual field test of standard winter wheat varieties regularly conducted at the college. This was effected by planting one-half (selected at random) of each plot with seed dusted with Ceresan plus phytohormone, and the other half with an equal quantity of seed of the same variety dusted with Ceresan (an organic mercury fungicide) only. Ten varieties were included in the test, each replicated six times, the field arrangement being in the form of six randomized blocks. The whole experiment thus comprised 60 main plots and 120 sub-plots. Main plots

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Contribution from the Department of Field Husbandry, Ontario Agricultural College, Guelph, and the Division of Biology and Agriculture, National Research Laboratories, Ottawa.
[2] *Professor of Field Husbandry, Ontario Agricultural College, Guelph.*
[3] *Statistician, National Research Laboratories, Ottawa.*
[4] *Chemist, National Research Laboratories, Ottawa.*

were 18½ ft. long and ten rows wide, the rows being spaced 6 in. apart. Sub-plots were 18½ ft. long and five rows wide. The soil on which the test plots were located was of the Miami loam group formed on a coarse glacial drift, and characterized by good natural drainage. The surface soil was neutral in reaction, rather low in available phosphate and from medium to low in potash. It was in excellent tilth, the result of plowing down a heavy stand of alfalfa earlier in the season.

It was desired to test ten different hormone preparations, so that the number of treatments was the same as the number of varieties. However, in order to provide some information respecting possible differential responses of varieties to treatment, each phytohormone dust was applied to three replicate plots of each of two varieties. The six randomized blocks were thus of two types, which may be designated *A* and *B*. Representing the ten winter wheat varieties, which will be found listed by name in Table II, by the Roman numerals I to X, and the ten hormone preparations by the Arabic numerals 1 to 10, then in the three blocks of type *A*, the phytohormones were applied to the wheats as follows:

I.1, II.2, ... IX.9, X.10,

whereas in the three blocks of type *B* the combinations were:

I.6, II.7, ... VI. 1,. . . . . X.5.

Ceresan was used throughout at the rate of ½ oz. per bushel, whilst the phytohormone dosages were 2, 5 and 10 p.p.m. of indolylacetic, 2, 5, 10, 20, 50 and 100 p.p.m. of naphthylacetic, and 2 p.p.m. of a mixture of indolyl- and naphthylacetic acids. In each case the hormone concentration is expressed as parts per million by weight of seed treated. The dusts were applied a few hours before planting, each lot of seed and dust being thoroughly shaken together in a wide-mouthed glass bottle in order to bring about as uniform a distribution of the dust as possible.

Seeding took place on September 2, 1937, at the rate of two bushels per acre, and harvesting on July 18, 1938. At harvest time, one foot at each end and the two outside rows of each sub-plot were discarded in order to minimize border or competitive effects.

Results

Field notes on germination and early growth were taken ten days after sowing, and although these were in some instances rather conflicting, on the whole there seems to have been a stimulation of vegetative growth by all four concentrations of indolylacetic acid, *viz.*, 2, 5 and 10 p.p.m., and 2 p.p.m. plus 2 p.p.m. naphthylacetic, and also by 2 and 5 p.p.m. of naphthylacetic; whereas the higher concentrations of naphthylacetic, namely 10, 20, 50 and 100 p.p.m., apparently retarded early development. Similar conclusions are to be deduced from visual estimates of the relative stand on the individual sub-plots subsequent to the resumption of growth in the spring, recorded on

May 6, 1938. On the other hand, the final height attained by the crop was but little affected, the only noticeable differences being an average increase of 2.3 in. in the sub-plots of Dawson's Golden Chaff receiving 5 p.p.m. of indolylacetic acid, and a decrease of 2.0 in. in those of Red Rock receiving 100 p.p.m. of naphthylacetic. In neither case was there any corresponding effect on the second variety receiving the same treatment.

There was no actual lodging in any of the sub-plots, but a certain amount of leaning was in evidence in some varieties which, however, never affected more than 20% of the crop. This tendency was slightly accentuated in the sub-plots receiving the lower concentrations (2 and 5 p.p.m.) of indolylacetic acid.

After harvesting, the separate weights of straw and grain, and the weight per measured bushel of the grain from the individual sub-plots were recorded, after which quarter-pound samples of the grain from each sub-plot were forwarded to the National Research Laboratories, Ottawa, for determination of nitrogen content by the Kjeldahl method. The data thus secured were subjected to analyses of variance, with the results indicated in Table I. Since the experiment was of the split-plot type, the quantities thus analyzed were not the actual yields, nitrogen contents, etc., but the differences in these respects between the hormone-treated and untreated halves of each main plot, hereafter on occasion designated "responses". It should be mentioned therefore that the season proved to be a favorable one, and that the outturn of crop was good, the average yield of grain for the experiment as a whole being 48.5 bu. per acre, the average yield of straw 6866 lb. per acre, the average weight per bushel of the grain, 61.2 lb., and its average nitrogen content 2.20% (dry basis).

It will be observed from Table I that there were significant differences between the hormone-treated and untreated sub-plots in respect of all four

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF WINTER WHEAT VARIETIES TO PHYTOHORMONE DUSTS

Source of variance	Degrees of freedom	Mean square			
		Straw production (000 omitted)	Grain yield	Weight per bushel	Nitrogen content
Between blocks:					
Between block types (1 d.f. interaction confounded)	1	5097	182.25*	0.87	.0216
Within block types (error (1))	4	*2604*	*20.91*	*0.69*	*.0282*
Within blocks:					
Average response	1	95600**	93.08*	3.95**	.0482*
Treatment differences	9	10923*	45.44*	0.82	.0234*
Interaction variety × treatment	9	3255	33.80*	0.69	.0075
Residual error (error (2))	36	3823	15.63	0.40	.0092

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*

TABLE II

RESPONSE OF WINTER WHEAT VARIETIES TO PHYTOHORMONE DUSTS

Phytohormone treatment	Variety to which applied		Straw production, lb./acre	Grain yield, bu./acre			Wt. per bushel, lb.	Nitrogen content (dry basis), %
	Blocks A	Blocks B		Blocks A	Blocks B	Average		
2 p.p.m. indolylacetic	D.G.C., O.A.C. 61	O.A.C. 104	+1174*	+3.88	+5.11*	+4.50	+0.7*	−.01
5 p.p.m. indolylacetic	Dawson's Golden Chaff	Dawtas	+ 702*	+1.76	+1.22	+1.49	+1.0*	−.06
10 p.p.m. indolylacetic	D.G.C., O-24-12	Imperial Amber	+ 722*	+6.80*	+0.32	+3.56	+0.4	−.04
2 p.p.m. naphthylacetic	Dawbul	Red Rock	+ 554*	+3.15	+1.73	+2.44	+0.2	−.10*
5 p.p.m. naphthylacetic	Junior No. 6	Kharkov	+ 358	+1.05	+2.28	+1.66	−0.2	−.04
10 p.p.m. naphthylacetic	O.A.C. 104	D.G.C., O.A.C. 61	+ 187	+2.84	−5.21*	−2.38	+0.2	+.05
20 p.p.m. naphthylacetic	Dawtas	Dawson's Golden Chaff	+ 424	+5.39*	−3.07	+1.16	0.0	+.08*
50 p.p.m. naphthylacetic	Imperial Amber	D.G.C., O-24-12	− 164	−4.62*	−5.06*	−4.84*	−0.2	−.12*
100 p.p.m. naphthylacetic	Red Rock	Dawbul	− 267	+5.90*	−5.95*	−0.02	+0.2	+.02
2 p.p.m. ind. + naphthylacetic	Kharkov	Junior No. 6	+ 302	+3.74	+3.65	+3.69	+0.1	−.05
Necessary for 5% level of significance			505	4.56	4.56	4.74	0.7	.08
Average			+ 399*	+2.99*	−0.49	+1.25*	+0.26*	−.028*

* *Individually significant, 5% level.*

characters dealt with. The results would thus seem to demonstrate the undoubted physiological activity of the chemicals tested when applied in this way, as well as the existence of some differences in response to the individual treatments. They do not, however, permit of any sharp discrimination between chemicals or dosages, as will be apparent from the necessary differences given in Table II. To do this would require a considerably higher degree of replication, since the intrinsic accuracy of this particular trial was about the average for field experiments of this type, the standard error of a single sub-plot in respect of grain yield, for example, being 2.8 bu. per acre, or 5.8% of the mean. In the case of grain yield, moreover, the situation is further complicated by the significant interaction, *i.e.*, differential response of varieties to the same treatment, indicated in Table I.

Table II lists the varieties to which each phytohormone preparation was applied in the blocks of type *A* and *B*, and also shows the effect of each, as estimated from the differences between adjacent treated and control sub-plots, on the yield of straw and on the yield, weight per bushel, and nitrogen content of grain. In the case of grain yield, which was shown in Table I to be differentially affected by the same treatment when applied to different varieties, the results for each variety (average of three main plots) are included separately. As, however, the effects on the other three quantities did not exhibit any significant differences between varieties, the mean for both varieties (average of six main plots) is given in these cases.

Significantly increased straw production is to be noted in the sub-plots receiving all three concentrations of indolylacetic acid alone, the most pronounced effect being secured from 2 p.p.m. In this respect, indolyl- proved to be more effective than naphthylacetic acid, from which only one significant response was secured, at a dosage of 20 p.p.m. In no instance, however, was the final yield of straw significantly depressed by treatment with either substance. It would seem that, in the absence of any pronounced differences in height, increases in straw production must be attributed to a denser stand. This might result either from an improvement in germination or survival, or from additional tillering. It has already been noted that some such differences in stand were apparent to visual inspection.

The effects on grain yield are not as clear-cut, owing to the differential response of varieties to the same treatment. Such differentials are perhaps not altogether surprising, in view of similar effects noted in fertilizer trials, but it is believed that this is the first occasion on which they have been demonstrated in an experiment with phytohormones on varieties of the same plant species. In one instance this resulted in a complete reversal, 100 p.p.m. of naphthylacetic acid increasing the yield of the variety Red Rock by 5.90 bu. per acre, but simultaneously depressing the yield of Dawbul by 5.95 bu.

Although less effective than indolylacetic in modifying straw production, naphthylacetic acid had more influence on grain yield, six significant differences being noted in individual varieties. Four of these, however, were depressive at the concentrations used (10, 50 and 100 p.p.m.). Significant increases were also obtained from 2 and 10 p.p.m. of indolylacetic acid, but

in both cases this response was forthcoming from only one of the two varieties thus treated.

It is of interest to note that kernel size, as indicated by the weight per measured bushel, was also apparently slightly affected. Over the entire experiment, there is a small but significant difference of 0.26 lb. per bushel in favor of the hormone-treated sub-plots. However, this seems to be almost entirely attributable to the indolylacetic acid treatments, as the differences for naphthylacetic fail to attain significance either individually or collectively. The effect is in no case pronounced, the maximum increase of 1.0 lb. (5 p.p.m. indolylacetic) being only about 2% in excess of the corresponding control.

On the whole, the effect of the phytohormones has been slightly to depress nitrogen content, although the average difference, 0.028%, is only 1.3% of the mean of the control sub-plots (2.21%). The maximum difference, −0.12% at 50 p.p.m., is of the order of 5% of the control. The grain from all three indolylacetic acid treatments is lower in nitrogen content than that of the controls, but none of these differences is statistically significant. Both increases and decreases in nitrogen content are in evidence in the naphthylacetic acid plots, although the positive effects are associated with phytohormone concentrations that probably would not be employed in normal practice, and that may well have affected the absorptive capacity of the root system. The average result is in accordance with expectation, and may perhaps even be regarded as providing independent evidence of real effects on yield, since if nitrification proceeded at substantially the same rate in the treated and untreated sub-plots, the nitrogen absorbed by the higher-yielding plants on the former would have to be distributed over more straw and grain. On the other hand, even after the exclusion of the higher dosages, there seems to be no consistent relation between the differences in nitrogen content and those in grain yield. As was pointed out previously, however, comparisons between treatments are subject to relatively large experimental errors. Furthermore, the recorded yields may be affected by accidents of harvesting, handling, etc., of which the nitrogen content would be largely independent.

As was suggested earlier in this section, the foregoing results, taken collectively, would seem to demonstrate the undoubted physiological activity of the phytohormone dusts used, under the conditions of this experiment. At the same time, it is clear that further experimentation with respect to both dosage and varietal characteristics, as well as possible seasonal effects, will be necessary before general conclusions can be reached. In the meantime, however, it may be of interest to observe that the maximum apparent stimulation of grain yield of an individual variety, namely 6.80 bu. (10 p.p.m. indolylacetic acid on Dawson's Golden Chaff 0–24–12), is an increase of approximately 13% over the control, and that the maximum observed increase in straw production, 1262 lb. (2 p.p.m. indolylacetic on Dawson's Golden Chaff, O.A.C. 61), represents an increment of roughly 20%.

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

V. DOUBLING THE CHROMOSOME NUMBER IN *T. VULGARE* AND F_1 OF *T. VULGARE* × *A. GLAUCUM* BY TEMPERATURE TREATMENTS[1]

By F. H. Peto[2]

Abstract

An apparatus was developed for applying heat treatments to spikes on plants growing in the field. This apparatus gave very satisfactory results on self-fertilized zygotes of Marquis wheat. Treatments at 42, 43, and 44° C. for 20 min. induced chromosome doubling in 2% of the plants.

A wide variety of temperature treatments was applied to more than 13,000 wheat florets 16 to 27 hr. after being pollinated with *A. glaucum*, in an attempt to produce fertile and stable F_1 hybrids. Chromosome doubling was induced in one F_1 plant of Kharkov × *A. glaucum* by exposure to alternating temperatures of 36° and 109° F. The resulting 84-chromosome plant grew slowly and failed to produce any spikes, as was the case with a number of 42-chromosome plants of this cross. Consequently there is uncertainty as to whether this plant is inherently abnormal aside from chromosome doubling, or whether the chromosome number is too high for normal development. It must be concluded from the results as a whole, that chromosome doubling by means of temperature treatments can be induced only with great difficulty in *Triticum–A. glaucum* hybrids.

Introduction

A. glaucum ($2n = 42$) crosses readily with both tetraploid and hexaploid species of wheat, but the F_1 generation is completely sterile, although back-crosses to the wheat parent have been made (1, 4). Since the F_1 plants of several of these crosses exhibit very desirable forage qualities, the problem of inducing fertility has received considerable attention.

Previously reported cytological studies (8) have shown that partial homology exists between one set of chromosomes from each of the parents with usually less than seven bivalents found at first metaphase. In addition, the chiasmata frequency per bivalent was found to be much lower in the hybrids than in the male parent *A. glaucum*, a situation that indicates a lower degree of homology among the pairing chromosomes in the hybrids. The meiotic irregularities caused by random behavior of numerous univalents are undoubtedly largely responsible for pollen degeneration and failure of anther dehiscence. However, if the chromosome number in these hybrids could be doubled, each of the chromosomes would then have a homologue with which it could pair. This should result in normal meiotic behavior and thereby overcome the main obstacle to fertility. Examples of the fortuitous doubling of the chromosome number in sterile F_1 hybrids are numerous. Winge (12) mentions 24 cases in which fertile and constant species hybrids have originated through doubling of the chromosome number. Since fertile and constant intergeneric hybrids have origininated through chromosome doubling of sterile

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. This contribution forms part of a co-operative investigation on the hybridization of Triticum and Agropyron, undertaken by the Dominion Experimental Farms and the National Research Council of Canada.

[2] *Cytogeneticist, National Research Laboratories, Ottawa.*

F_1 hybrids such as *Aegilops ovata* ($2n = 28$) × *Triticum durum* ($2n = 28$) and *Triticum vulgare* ($2n = 42$) × *Secale cereale* ($2n = 14$), it is reasonable to expect similar results from doubling the chromosome number of *Triticum–A. glaucum* hybrids.

Randolph (10) was one of the first workers to utilize heat treatments in producing new tetraploid strains. In 1932 he was successful in inducing chromosome doubling in maize by applying heat locally to ears of corn at the time of the first zygotic division. The chief advantage of Randolph's method was that if doubling occurred at this stage the resulting plants were completely tetraploid. The author's interest in the possibilities of utilizing heat treatments to induce chromosome doubling originated from studies begun in 1933 on the influence of temperature on the mutation rate in barley (7) when tetraploid sectors in root tips of barley were observed after heat treatments of germinating barley seed. In one case a partially tetraploid barley spike was discovered in a plant that had been subjected to a temperature of 35° C. for seven days during germination (9). Attempts to duplicate these results were unsuccessful and Randolph's time of treatment was finally adopted. The effectiveness of heat treatments during the early zygotic divisions in inducing completely tetraploid plants has been recently demonstrated by several workers. Atwood (2) reported in 1936 the occurrence of a fertile tetraploid plant of *Melilotus alba* from local heat treatment of the racemes at 40 to 41° C. for 30 min. Müntzing, Tometorp and Mundt-Petersen (6) found nine barley plants with chromosome numbers from 27 to 30 in the second generation after the local application of heat treatments at 45° C. for 30 min. Dorsey (3) was successful in inducing chromosome doubling in *Triticum vulgare*, *Secale cereale* and in sterile F_1 hybrids of *T. vulgare* × *S. cereale*. The amphipolyploid wheat-rye plant was partially fertile. The meiotic behavior of this plant was not investigated but it is reasonable to assume that normal bivalent formation would occur.

Apparatus and Methods

An apparatus for applying heat treatments in the field was built and three units in operation are shown in the photograph in Plate I, Fig. 1. The exterior of the water jacket is 18 in. high and 5½ in. in diameter and constructed of 16-gauge copper. The treatment chamber proper is constructed from a 14-gauge brass tube 1½ in. in diameter and 16 in. long. This tube was soldered in position in the centre of the outer chamber with the lower end left open so that the apparatus can be slipped over the spike to be treated. A ½-in. brass tube 3 in. in length was soldered to the upper end of the 1½ in. tube, so that the bulb of a sensitive thermometer could be placed in close contact with the spikes during treatment. A DeKhotinsky thermoregulator was used to control the temperature of the water in the water jacket. Heat was applied by resistance wire wound around the lower third of the jacket's outer surface, which was suitably insulated with asbestos tape. Heat losses to the exterior were reduced by ½-in. felt held in place by cotton lagging. The

apparatus was supported on a tripod having a large base 5½ in. in diameter with a 2½ in. hole in the centre to allow the spikes to slip into the inner tube of the heater. Tests were made on the temperature variations within the treatment chamber with the bottom open. The air temperature in the portion of the treatment chamber 4 in. or more above the opening could be maintained within ±0.5° C. of the desired temperature of 40 to 48° C. In field tests it was found that two spikes could be treated at the same time without any significant depression of the air temperature at the beginning of treatment.

In 1936, the heat treatment apparatus was used exclusively on plants growing in the field, but in 1937 the experiments were modified to include low- as well as high-temperature treatments, since Sax (11) obtained much better results by this method. The plants to be hybridized and treated were grown in the field in 6-in. pots, which in turn were embedded in soil. Growth under these conditions resembled closely that of plants growing free in the soil and it was possible to remove without damage the potted plants for the duration of the treatment. The low-temperature treatments were carried out by transfer of the plants to a refrigerator room at 36° F. The high temperature treatments were carried out in small greenhouse rooms in which the temperature could be controlled within ±1° F. at any required temperature between 95 and 109° F. A very high humidity was maintained in these rooms by spraying the floors and moistening the peat on the benches prior to treatment.

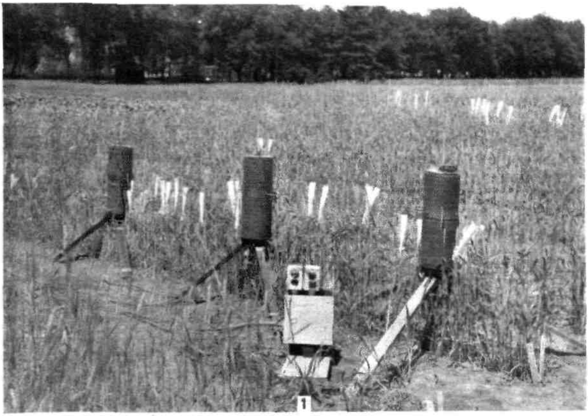
The method of emasculating the wheat florets and pollination with *A. glaucum* was similar to that described by Johnson and McLennan (5). The various treatments were begun 16 to 27 hr. after pollination, with narrower limits in certain experiments.

Heat Treatments of Marquis Wheat

The objects of these preliminary treatments were to determine the optimum conditions for inducing chromosome doubling in the early zygotic divisions after natural self pollination, and to determine the effects of the various treatments on fertility. Twenty-seven spikes with 50% or more florets in flower were selected. The central florets were removed and the total number of florets reduced to 20. The time of flowering was noted, and nine spikes were treated after elapsed periods of 16, 20 and 24 hr., respectively. In each case, three spikes were heat-treated for 20 min. with the field heat treatment apparatus at each of the temperatures 42, 43, and 44° C.

The data from this experiment are given in Table I. The percentage seed set was decreased with increase in treatment temperature and with decrease in the period between pollination and treatment. These results indicate that the treatment temperatures, duration of treatments and time lapse after flowering were in the desired critical range in which chromosome doubling might be expected without complete inhibition of seed formation.

From the heat-treated zygotes of Marquis, 295 plants were grown and carefully examined during early growth. Ten plants were noticeably different



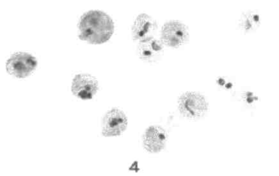


FIG. 1. *Apparatus for heat treatment in operation.* FIG. 2. *84-chromosome plant of Marquis wheat.* FIG. 3. *42-chromosome plant of Marquis wheat.* FIG. 4. *Pollen from 84-chromosome plant of Marquis wheat.* FIG. 5. *Pollen from 42-chromosome plant of Marquis wheat.*

from the others. They had a slower growth rate, the leaves appeared to be coarser and sometimes irregular in shape, and the stomata seemed larger than normal. Chromosome counts were made on nine of these plants; six plants had 84 chromosomes or double the normal chromosome number, and the remainder had the normal complement of 42 chromosomes. The 84-chromosome plants grew more slowly than the 42-chromosome plants, reaching the flowering stage about a month later under winter greenhouse conditions.

TABLE I

RESULTS FROM HEAT TREATMENTS OF MARQUIS WHEAT

Time after flowering, hr.	Temp., °C.	No. florets pollinated	No. seeds set	Seed set, %	Seed set, mean %
16	42	60	35	58.3	
16	43	54	22	40.7	46.5
16	44	60	24	40.0	
20	42	56	31	55.4	
20	43	58	36	62.0	57.0
20	44	58	31	53.4	
24	42	60	49	81.7	
24	43	60	36	60.0	66.1
24	44	60	34	56.7	

Percentage seed set at 42° = 65.1
Percentage seed set at 43° = 54.2
Percentage seed set at 44° = 50.3

They, however, eventually attained normal height, tillered abundantly and seemed slightly stouter and coarser than the normal plants. All the plants were sterile, as the anthers failed to dehisce. Pictures of 84- and 42-chromosome plants are shown in Plate I, Figs. 3 and 4. Chromosome doubling is usually disadvantageous in a highly fertile pure species with a high polyploid chromosome number, consequently the unfavorable results obtained with the 84-chromosome plants were not surprising. The experiment was, however, successful as a further demonstration of the practicability of the heat treatment method for inducing autopolyploid forms, and gave encouragement to the efforts to induce chromosome doubling in sterile *Triticum–A. glaucum* hybrids.

Comparison of pollen grains in 42- and 84-chromosome wheat plants

Ripe anthers were fixed in absolute alcohol, three parts, and glacial acetic, one part, and transferred to 70% alcohol. They were mounted and stained in aceto-carmine. Measurements were taken with a micrometer eyepiece, one unit of the recorded measurement being 15 μ. The data given in Table II show that the pollen of the 84-chromosome plant was about 5.5μ larger on the average. The values given for the standard deviation of the mean show that the pollen of the 84-chromosome plant was much more variable. The

reasons for this situation were apparent when pollen development was studied. The data on these studies are given in Table III, and pollen photographs are shown in Plate I, Figs. 4 and 5. All the pollen grains of the 42-chromosome form were normal in that the vegetative and two sperm nuclei were always present and the grains were filled with cytoplasm. Development of pollen in the 84-chromosome plants was very irregular or had been retarded at various stages. For example, only 48.5% of the grains possessed three nuclei and an equal percentage possessed only vegetative and generative nuclei. There was a reduced amount of cytoplasm in 30% of the pollen grains of this plant, and 11.5% contained only a slight amount of cytoplasm. The anthers failed to dehisce on account of these abnormalities, and the 84-chromosome plants were therefore completely sterile.

TABLE II

MEASUREMENTS OF POLLEN DIAMETER

—	Mean diameter (μ)	S.D. of the mean
T. vulgare (42-chromosome plant)		
Sample 1	56.9	0.40
Sample 2	56.1	0.48
	Mean 56.5	
T. vulgare (84-chromosome plant)		
Sample 1	60.4	0.65
Sample 2	63.4	1.02
	Mean 61.9	

TABLE III

POLLEN DEVELOPMENT IN THE 42- AND 84-CHROMOSOME FORM OF *T. vulgare*

Plants	Cytoplasm	Nuclei 3	Nuclei 2	Nuclei 1	Nuclei 0	Total	Per cent
Normal $2n = 42$	Normal	200	0	0	0	200	100
	Reduced	0	0	0	0	0	0
	Slight	0	0	0	0	0	0
	None	0	0	0	0	0	0
	Total	200	0	0	0		
	Per cent	100	0	0	0		
Mutant $2n = 84$	Normal	58	50	3	0	111	55.5
	Reduced	24	35	1	0	60	30.0
	Slight	11	10	2	0	23	11.5
	None	4	2	0	0	6	3.0
	Total	97	97	6	0		
	Per cent	48.5	48.5	3.0	0		

Stomatal measurements

The average over-all length and width of the guard cells of the leaves from 42- and 84-chromosome plants are given in Table IV. The mean stomatal

length and width of the 84-chromosome plant exceed those of the 42-chromosome plant by 9.5 and 4.5μ respectively. These differences represent increases of about $\frac{1}{7}$, whereas increases of about $\frac{1}{4}$ in linear dimensions would be expected if chromosome doubling had doubled cell volume. The differences between the plants showed up more clearly in the product of the length and width which should give a single value bearing a close relation to volume.

TABLE IV

COMPARSION OF STOMATAL SIZES IN 42- AND 84-CHROMOSOME PLANTS

Plant	Chr. no. (2*n*)	No. stomata examined	Mean length (μ)	Mean width (μ)	Mean product L × W (sq. μ)
Marquis	42	100	70.0	30.9	2,174
Marquis	84	100	79.5	35.4	2,836
F_1 Kharkov × *A. glaucum* (121–4)	42	50	59.9	33.9	2,036
F_1 Kharkov × *A. glaucum* (121–5)	42	50	52.4	33.8	1,773
*F_1 Kharkov × *A. glaucum* (121–1)	84	100	65.2	43.5	2,840
*F_1 Kharkov × *A. glaucum* (121–1)	84	50	57.4	40.8	2,347

* *Individuals of the same clone.*

If accurate estimates of volume could have been secured, it seems probable that the differences would have been even more pronounced. Considering, however, the small mean differences between the linear dimensions of the 42- and 84-chromosome plants and the wide variation between individual measurements, *it is apparent that any superficial stomatal observations* would be of little value in detecting 84-chromosome plants.

Temperature Treatments to Obtain Amphipolyploid *Triticum-A. glaucum* Hybrids

Experiments during 1936

A total of 5140 florets of the various tetraploid and hexaploid species and varieties of *Triticum* (Table V) were pollinated with *A. glaucum* and subjected to heat treatments with the portable apparatus 18 to 27 hr. later. The three apparatuses were set at temperatures of 42, 43 and 44° C. and the duration of treatment was 20 min. in each case. A summary of the results is given in Table V. The data for the three treatment temperatures are combined in the summary, as there were no clear-cut differences. From the 5140 florets treated, only 111 seeds were obtained, seed set in individual crosses varying from 0 to 5.5%. The germinability of the seed was rather low, as only 50 seedlings were obtained. Chromosome counts made on the root tips of these seedlings showed all to possess the undoubled chromosome number.

A partial explanation of the failure to double the chromosome number is undoubtedly the fact that only approximately 1% of the fertilized florets produced viable hybrid plants. Assuming that the frequency of induced

chromosome doubling observed in self-fertilized Marquis (2%) would apply to the hybrids, then approximately 2% of the plants from treated zygotes would be expected to have the doubled chromosome number. Consequently

TABLE V

SUMMARY OF 1936 HEAT-TREATMENT RESULTS FROM CROSSES BETWEEN *Triticum* AND *A. glaucum*

Female parent	No. florets treated	No. seeds	Per cent seed set	No. seedlings
T. vulgare var. Lutescens	1780	36	2.0	10
T. vulgare var. C.A.N. 1835	1060	7	0.7	0
T. vulgare var. Marquis	600	17	2.8	4
T. vulgare var. Canus	280	0	0	0
T. durum var. Mindum	840	46	5.5	33
T. turgidum	340	2	0.6	2
T. persicum var. Black Persian	180	3	1.7	1
T. dicoccum var. Vernal	60	0	0	0
Totals	5140	111	2.2	50

it would be necessary to treat over 5000 florets to expect even one hybrid plant with the doubled chromosome number. Since only slightly more than this number were treated, the failure in this year's experiments to obtain amphipolyploid seedlings is not surprising.

Experiments during 1937

The heat-treatment technique employed in 1936 was modified in 1937 to include alternating low and high temperatures. A wide variety of treatments was applied as shown in Tables VI and VII. It was impossible to have enough florets of any combination ready for treatment at one time to give a completely satisfactory test of any given treatment condition, since the percentage success of any treatment, as regards chromosome doubling, was expected to be very low, judging from the results of previous experiments. It was thought, therefore, that the chances of producing an amphipolyploid hybrid would be greater if a wide range of conditions were tried than if one or two treatment conditions were arbitrarily selected and thoroughly tested.

The winter wheat varieties, Dawson's Golden Chaff and Kharkov, and the spring wheat varieties, Lutescens, Marquis and Vernal emmer, were used as the female parents and pollinated with *A. glaucum*. The method of procedure in a typical treatment was as follows: The plants on which the florets had been pollinated the previous day were removed from the field at 9 a.m. and placed in the cold room at 36° F. At 9 p.m. that night these plants were transferred to the greenhouse room at 95° F. At 9 a.m. the following day they were replaced in the cold room at 36° F. and removed at 9 p.m. that night.

In certain of the treatments the field heat-treatment apparatus was also used. Following these treatments the plants were, in several experiments, transferred to the cold room at 36° F. for 10 hr.

TABLE VI

SUMMARY OF 1937 TEMPERATURE-TREATMENT RESULTS FROM CROSSES BETWEEN WHEAT AND *A. glaucum*

Temperature treatment, °F.	Female parent	Leaf and stem injury after treatment	No. florets pollinated	No. of seeds		Hybrid seed set, %	No. plants
				Prob. hybrids	Prob. selfs		
3 hr. 36°	Dawson's G.C.	0	392	11	2	2.8	7
+ 3 hr. 104°	Marquis	2	20	0	0	0	0
+ 6 hr. 36°	Lutescens	1–3	60	0	0	0	0
+12 hr. 104°	Vernal	0–1	140	1	4	0.7	0
+ 3 hr. 36°							
			612	12	6	2.0	7
3 hr. 36°	Dawson's G.C.	0–1	168	26	0	15.5	18
+ 3 hr. 109°	Kharkov	0–1	224	37	0	16.5	5*
+ 3 hr. 36°	Marquis	1–4	140	0	0	0	0
+ 3 hr. 109°	Lutescens	2–3	80	0	2	0	0
			612	63	2	10.3	23
1.5 hr. 36°	Dawson's G.C.	1	28	0	0	0	0
+2.5 hr. 111°	Kharkov	1	56	0	0	0	0
+8 hr. 36°	Lutescens	2	20	1	0	5.0	0
	Vernal	0–1	140	20	0	14.3	15
			244	21	0	8.6	15
6 hr. 36°	Dawson's G.C.	1	112	1	0	0.9	1
+ 6 hr. 104°	Marquis	0–1	80	0	0	0	0
+12 hr. 36°	Vernal	1–2	60	0	0	0	0
			252	1	0	0.4	1
12 hr. 36°	Marquis	2–4	360	27	4	7.5	3
12 hr. 104°	Lutescens	3–4	300	17	0	6.0	9
	Vernal	3–4	120	0	0	0	0
			780	44	4	5.8	12
12 hr. 36°	Dawson's G.C.	0	96	0	0	0	0
+12 hr. 95°	Marquis	0–1	280	34	0	12.1	2
+12 hr. 36°	Lutescens	0–1	520	28	11	5.4	4
	Vernal	0–1	180	70	0	38.9	57
			1076	132	11	12.3	63
6 hr. 36°	Marquis	0–1	120	50	0	41.7	11
+6 hr. 104°	Lutescens	0–1	140	66	0	47.1	15
	Vernal	0–1	180	92	0	51.1	75
			440	208	0	47.3	101
6 hr. 36°	Marquis	0–1	40	0	0	0	0
+4 hr. 104°	Lutescens	1–2	80	7	0	8.7	2
	Vernal	0–1	80	23	7	28.7	21
			200	30	7	15.0	23

**The 84-chromosome plant was found in this lot of plants.*

TABLE VI—*Concluded*

SUMMARY OF 1937 TEMPERATURE-TREATMENT RESULTS FROM CROSSES BETWEEN WHEAT AND *A. glaucum*—*Concluded*

Temperature treatment, °F.	Female parent	Leaf and stem injury after treatment	No. florets pollinated	No. of seeds		Hybrid seed set, %	No. plants
				Prob. hybrids	Prob. selfs		
6 hr. 36° +2 hr. 104°	Dawson's G. C	1	56	0	0	0	0
	Marquis	0–1	40	11	0	27.5	3
	Lutescens	1–2	60	7	0	11.7	7
	Vernal	0	20	1	0	5.0	1
			176	19	0	10.7	11
6 hr. 36° +90 min. 105°	Marquis	1	40	1	0	2.5	0
	Lutescens	0–1	60	1	0	1.7	1
			100	2	0	2.0	1
6 hr. 36° +60 min. 105°	Marquis	1	20	2	0	10.0	0
	Lutescens	0–2	100	6	6	6.0	6
			120	8	6	6.7	6
6 hr. 36° +30 min. 105°	Marquis	1	40	0	0	0	0
	Lutescens	1	40	0	0	0	0
	Vernal	0	40	7	2	17.5	7
			120	7	2	5.8	7
6 hr. 36° +90 min. 104°	Marquis	0–1	80	2	0	2.5	0
	Lutescens	1	40	2	7	5.0	0
			120	4	7	3.3	0
6 hr. 36° +60 min. 104°	Marquis	1	180	29	0	16.1	7
	Lutescens	0–1	160	5	1	3.1	1
			340	34	1	10.0	8
6 hr. 36° +30 min. 104°	Marquis	0	20	6	0	30.0	0
	Lutescens	0–1	100	19	1	19.0	2
			120	25	1	20.8	2
12 hr. 36° +12 hr. 95°	Marquis	0–4	400	10	2	2.5	1
	Lutescens	0–3	180	3	0	1.7	0
			580	13	2	2.2	1

When the whole plant was subjected to high temperatures, the more severe treatments caused extensive injury to the leaves and exposed leaf sheaths. Notes on this injury were taken and the plants graded from 0 to 5, the higher grades indicating the more severe injury. The range of injury for each treatment is given in Table VI. Foliage injury did not always inhibit seed development, since a number of hybrid seeds were set following severe injury from treatment at 104° F. for 12 hr.

TABLE VII

Temperature treatment		Female parent	No. florets	No. seeds		Hybrid seed set, %	No. plants
Field apparatus, °C.	Cold room, °F.			Prob. hybrids	Prob. selfs		
20 min. 42°	10 hr. 36°	Dawson's G.C.	112	0	0	0	0
20 min. 46°	10 hr. 36°	Dawson's G.C.	104	0	0	0	0
			216	0	0	0	0
20 min. 42°	10 hr. 36°	Marquis	220	12	0	5.5	1
20 min. 43°	10 hr. 36°	Marquis	240	6	0	2.5	0
20 min. 44°	10 hr. 36°	Marquis	60	7	0	11.7	0
20 min. 45°	10 hr. 36°	Marquis	40	0	0	0	0
20 min. 46°	10 hr. 36°	Marquis	100	0	0	0	0
			660	25	0	3.8	1
20 min. 42°	10 hr. 36°	Lutescens	120	3	1	2.5	0
20 min. 43°	10 hr. 36°	Lutescens	160	3	0	1.9	0
20 min. 46°	10 hr. 36°	Lutescens	100	5	2	5.0	3
			380	11	3	2.9	3
20 min. 42°	10 hr. 36°	Vernal	80	4	0	5.0	4
20 min. 43°	10 hr. 36°	Vernal	20	1	0	5.0	1
20 min. 46°	10 hr. 36°	Vernal	60	0	0	0	0
			160	5	0	3.1	5
20 min. 43°		Marquis	60	0	0	0	0
20 min. 44°		Marquis	20	0	0	0	0
			80	0	0	0	0
20 min. 42°		Lutescens	20	2	0	10.0	0
20 min. 45°		Lutescens	120	0	0	0	0
20 min. 46°		Lutescens	140	0	0	0	0
20 min. 48°		Lutescens	40	0	0	0	0
			320	2	0	0.6	0
20 min. 42°		Vernal	20	0	0	0	0
20 min. 44°		Dawson's G.C.	140	0	1	0	0
20 min. 45°		Dawson's G.C.	84	0	0	0	0
			224	0	1	0	0
Untreated check		Dawson's G.C.	28	1	0	3.6	1
Untreated check		Kharkov	28	1	0	3.6	0
Untreated check		Marquis	60	4	1	6.7	2
Untreated check		Lutescens	140	4	0	2.9	0
			256	10	1	3.9	3

The most striking feature of the results recorded in Tables VI and VII is the lack of correlation between severity of treatment and amount of seed set. However, the treatments are not strictly comparable as it was impossible to pollinate enough plants to enable more than two or three treatments to

be carried out simultaneously. Consequently the following variables might influence the results: (1) stigmatic receptivity; (2) viability of pollen; (3) influence of weather on pollen tube growth rate.

The seed set following heat treatment with the apparatus in the field was lower than that for similar heat treatments carried out in the greenhouse. For example, the apparatus treatment at 42° C. (107.6° F.) for 20 min. would be expected to be less severe than two three-hour periods at 109° F. in the greenhouse. However, the amount of seed set from Dawson's Golden Chaff pollinated with *A. glaucum* was 15.5% after the latter treatment, and none was set after the former. The additional severity of the apparatus treatments may be the result of very rapid heat transfer from contact of the spike with the inner surface of the metal tube, and also because of the lower humidity of the air surrounding the spike.

TABLE VIII

POLLINATION, SEED, AND GERMINATION DATA FOR VARIETIES OF WHEAT POLLINATED WITH *A. glaucum* AND SUBJECTED TO VARIOUS TEMPERATURE TREATMENTS

Wheat variety	No. florets pollinated	No. hybrid seeds	Hybrid seeds, %	No. plants grown	Viability, %
Dawson's G.C.	1292	38	2.9	27	71.1
Kharkov	280	37	13.2	5	13.5
Marquis	2600	197	7.6	30	15.2
Lutescens	2640	176	6.7	50	28.4
Vernal (emmer)	1140	219	19.2	181	82.6
Total	7952	667	8.4	293	43.9

A total of 7952 wheat florets were pollinated with *A. glaucum* and subjected to various temperature treatments (Table VIII); 667 seeds were produced and 293 plants were grown. The viability of the seed of Dawson's Golden Chaff and Vernal hybrids was good, while that of the other hybrids was very poor. The percentage of hybrid florets producing seeds after temperature treatment was similar, on the average, to the results obtained by Armstrong (1) and Johnson (4) without heat treatment, with the exception that Johnson reports a much higher seed set for Dawson's Golden Chaff hybrids than that obtained in the present study. There is some evidence that moderate temperature treatments may greatly increase the hybrid seed set, because after a treatment of 6 hr. at 36° F. and 6 hr. at 104° F. the seed set for Marquis, Lutescens, and Vernal hybrids was 41.7, 47.1 and 51.1%, respectively. Johnson's results (4) for the same crosses were only 2.0, 2.5 and 6.4%, and Armstrong's results were 8.4% for Lutescens and 34.6% for Vernal hybrids.

Chromosome counts were made on the root tips of most of the 293 hybrid plants, which with one exception were found to possess the undoubled chromosome number. This exceptional plant was from a cross between Kharkov and *A. glaucum* and possessed 84 instead of 42 chromosomes. The treatment producing this amphipolyploid plant was one of the most severe applied.

It began about 18 hr. after pollination and was as follows: 9 to 12 a.m. at 36° F., 12 to 3 p.m. at 109° F., 3 to 6 p.m. at 36° F., and 6 to 9 p.m. at 109° F. A total of 224 florets were treated in this way and 26 hybrid seeds were obtained, from which five plants were grown. Two of these plants were very weak; one died in the seedling stage, and the other was so puny that root-tip counts could not be made. Chromosome counts were, however, made on the other three plants, two of which had 42 chromosomes and the other, as indicated, 84 chromosomes. One of the 42-chromosome plants (121–5) was rather slow growing and failed to produce any spikes, as was the case with the 84-chromosome plant, although the latter was slightly more vigorous. The other 42-chromosome (121–4) plant was very vigorous, produced an abundance of sterile spikes and in general resembled the better F_1 plants of this cross produced in previous years.

The failure of the 84-chromosome plant to produce any spikes prevented a meiotic study being made to determine whether chromosome doubling would result in normal bivalent formation. There are two possibilities that might account for the failure of this plant to develop normally. Either it is inherently abnormal, regardless of chromosome doubling, as is the case with a number of the 42-chromosome plants of this cross, or the chromosome number is too high for normal growth and differentiation. If the former is the case, it would be worth the effort to produce other hybrids with the doubled chromosome number. However, it should be kept in mind that the 84-chromosome wheats did not develop normally. It is therefore likely that better results might be expected from doubling the chromosome number in crosses between the tetraploid ($2n = 28$) wheats and *A. glaucum* which would give an amphipolyploid variety with 70 chromosomes.

A study was made on the relation of cell size in root tips to chromosome doubling. Comparable cross sections of the root tips of the 84-chromosome hybrid plant and of two 42-chromosome plants of the same cross were examined. The outlines of the areas in which the cell size was to be determined were drawn with the aid of a *camera lucida* and the area measured with a rotometer. The area of the cross section studied was a sector comprising one-quarter of the circumference which included the epidermal and the three outer endodermal layers. The number of cells in an area was counted and the average area per cell calculated. The approximate cell volume was calculated by cubing the square root value of the mean cell area. The data are shown in Table IX and clearly indicate that cell volume in root tips is directly proportional to chromosome number. The ratio in cell volume of 42- and 84-chromosome plants would be expected to be 0.5, whereas actual calculations showed it to be 0.525 and 0.559.

Stomatal sizes were also compared in the 42- and 84-chromosome F_1 plants of Kharkov × *A. glaucum* (Table IV). The same plants were studied as were used for the cell-size measurements in root tips, but the leaves were collected on one-year-old plants and the root tips were studied in the seedling stage. The cell size in the root tips was very similar in plants 121–4 and 121–5, but the stomatal size of the former was much larger. Plant 121–4 is much

more vigorous than 121–5 and this may partially account for the larger stomatal size. This, however, does not explain the differences in stomatal size between individuals of the same clone of the 84-chromosome plant (121–1) which should be equally vigorous. It is suspected that the slightly different stages of development at which the leaves were collected might account for this lack of agreement. In spite of the above variation, the mean product of stomatal length and width of the 84-chromosome individuals is greater than that of the 42-chromosome individuals. These data substantiate the conclusions drawn from observations on Marquis regarding the unreliability of stomatal measurements as a positive indicator of chromosome doubling.

TABLE IX

COMPARISON OF CELL SIZE BETWEEN 42- AND 84-CHROMOSOME HYBRIDS OF KHARKOV × *A. glaucum* (Magnification = 350×)

Plant No.	Chromosome number	Sector area, sq. in.	No. of cells	Cell area, sq. in.	Calculated cell volume, cu. in.	Ratio 42 chr./ 84 chr.
121–5	42	2.61 2.80 2.46 2.43	30 30 27 26	0.087 0.093 0.091 0.093		
			Average	0.091	0.0275	0.525
121–4	42	2.60 2.59 2.34 2.56	27 27 25 27	0.096 0.096 0.094 0.095		
			Average	0.095	0.0293	0.559
121–1	84	3.85 3.72 3.82 3.74	27 27 26 28	0.143 0.138 0.143 0.134		
			Average	0.140	0.0524	

Discussion

Heat treatments at 42, 43, and 44° C., applied to Marquis wheat 16 and 24 hr. after self-pollination, with the specially constructed heat-treatment apparatus caused the doubled chromosome number to occur in about 2% of the resulting plants. This method is therefore very satisfactory for inducing chromosome doubling in fertile, non-hybrid zygotes. However, when this method is applied to hybrid zygotes in which the percentage seed set is very low, the chances of inducing chromosome doubling are greatly reduced, as is seen from the treatments in 1936 of 5140 florets with negative results. Consequently, if chromosome doubling is to be induced by temperature treatments of a moderate number of hybrid zygotes, either the treatment methods must be improved, so that a chromosome doubling would occur in a much higher percentage of hybrid zygotes, or the present methods restricted

to hybrid combinations in which a high percentage seed set might be expected. In the 1937 experiments, high and low temperature treatments were alternated in the hope that this might prove a more effective method and, in addition, certain wheats were selected which were expected to set a high percentage of seed on being pollinated with *A. glaucum*. In spite of these improvements only one amphipolyploid plant was produced from 7952 florets pollinated. Peculiarly enough, this plant was produced under very severe treatment conditions in which only 224 florets of Kharkov pollinated with *A. glaucum* had been treated. It is possible that duplication of this treatment on a large scale on hybrid zygotes which set a high percentage of seed might yield satisfactory results. Nevertheless, it must be concluded that chromosome doubling by means of temperature treatments can be induced only with great difficulty in *Triticum–A. glaucum* hybrids.

The alkaloid colchicine has recently been found to be particularly useful in doubling chromosome numbers in plants. Studies in this laboratory have shown it to be much more effective with dicotyledonous than with monocotyledonous species. Nevertheless, the possibilities of using this drug to obtain amphipolyploid *Triticum–A. glaucum* hybrids is being thoroughly tested before further temperature treatments are undertaken.

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